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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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INHERITANCE OF CHLOROPHYLL CHARACTERS IN SORGHUM

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Received May 29, 1930

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INTRODUCTION

So many recessive chlorophyll characters among plants have been reported upon in recent years that the recorded instances of such defects are almost innumerable. These characters may be lethal in their effect upon the homozygote or affect only a reduction of the chlorophyll with consequent retarded development of the plant. Chlorophyll deficiencies have been found most frequently in maize, the most notable accounts being the eminent work of EMERSON, LINDSTROM, DEMEREC, and others in reporting upon the inheritance and linkage relations of this large group of characters. A few similar recessives have also been found in such crops as wheat, barley, rice, and cotton. They have not thus far been reported in sorghum, however, except in a preliminary report of the occurrence of white seedlings (CONNER and KARPER 1924), the inheritance of which is given here more fully.

It is the purpose of this paper to describe and report the mode of inheritance of the various chlorophyll seedling characters so far studied in sorghum. In 1923, when the occurrence of white seedlings in sorghum first came to our attention when working with some inbred lines of kafir, a further search for other similar characters was instituted. In recent years

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their appearance has been encountered so frequently as to resemble closely their common occurrence in maize. Although the problem has not been worked out covering the whole range of chlorophyll characters and their interrelation in sorghum, sufficient evidence is at hand to point to a parallelism with many of the chlorophyll deficiencies found in maize. Excepting maize, the seedling characters concerned with chlorophyll reduction so far found in sorghum are probably more extensive and numerous than those yet found in any other species. So far none have been found in sorghum that have not already been described and studied in maize, and it is believed that other seedling as well as mature plant chlorophyll characters which have been found in the latter species may also be found in sorghum. Striping of various types and other mature plant colors which have not yet been studied are found in sorghum similar in appearance to those in maize. While sorghum cross-fertilizes readily, it is naturally self-fertilized to the extent of about 94 percent. KARPER and CONNER (1919) found a wide variation in the amount of natural cross-pollination in sorghum, ranging from less than 2 percent to 35 percent, averaging 6.18 percent under favorable conditions for crossing. Following self-fertilization in kafir, however, it is interesting to note that out of eight pure lines selfed from year to year chlorophyll deficiencies have appeared in three of the lines during the first six years of inbreeding.

Whenever the same type of chlorophyll deficiency has been found from a distinctly different source, it has been designated by a different symbol, indicating a different factor pair. In such instances the designation is a tentative one and does not mean that these characters are genetically different but rather that proof of their being due to the same factor has not yet been established. Some of the interrelationships and linkages between the various characters are now being studied but the procedure and technique are much more complicated than a similar undertaking with maize and several years will be required to complete the task.

The material used, description, and history of the various chlorophyll deficiencies will be taken up separately under each of the characters studied.

WHITE SEEDLINGS

Genotype w_1w_1

This was the first chlorophyll character to be found in sorghum. It occurred in a single head row of Standard Blackhul kafir, Line 223, which had been inbred for six generations. The parent plant of the head row was heterozygous for white seedlings when first discovered and segregated in the initial trial in a ratio of 834 normal and 222 albino seedlings, or 3.75:1.

This character in sorghum is quite typical of the white seedlings found in other crops, the homozygous recessives being devoid of any chlorophyll and dying in the field at from a week to ten days after emergence. Albinos in sorghum are usually pure white instead of the creamy cast often found in maize.

Since the preliminary test of the inheritance did not give a very satisfactory ratio, 245 selfed plants were grown the following year from remnant seed of the original head. A summary of the breeding behavior of these progenies and the segregation of the heterozygous plants is given in table 1.

TABLE 1

Breeding behavior of 245 F_2 progenies of a plant heterozygous for white seedlings.

GENOTYPE	NUMBER OF PROGENIES		NUMBER OF SEEDLINGS		*Calculated White*	DEVIATION	RATIO
	OBSERVED	*Calculated*	GREEN	WHITE			
Ww	147	*163*	8116	2793	*2727.25*	65.75±30.51	2.91:1
WW	98	*82*	All				

The proportion of segregating to non-segregating progenies was not in close agreement with the expected proportions of 2:1, the deviation being 16±4.98 and the odds about 31:1. Practically all of the 147 heterozygous plants, however, showed a seedling ratio deviating only slightly from the expected ratio on the basis of a single factor. Only five of the deviations were as much as three times their probable error. The segregation of 2.91:1 indicates a slight deficiency of green seedlings, while in the parent head there was an excess of greens. The complete results, however, are in close agreement with the expected Mendelian ratio where a single gene is involved so that the w_1 chlorophyll character may be regarded as simple in its inheritance.

Genotype w_2w_2

The w_2 factor was also derived from inbred line No. 223 of Blackhul kafir. White seedlings having been completely eliminated from this stock in 1924, it was free from chlorophyll deficiencies until 1928 when three of the ten head rows planted to this line proved to be segregating for albinos. This character may be identical to that produced by the w_1 factor and may be merely a new mutation of the same gene. The stock had been inbred for twelve generations when this character appeared. The mutation probably occurred at gametogenesis in 1926.

Twenty plants were grown from a segregating head in 1929 for a study

of the progeny in the greenhouse. Six of them proved to be homozygous green and fourteen heterozygous for white seedlings. Data on the segregation of the latter are given in table 2.

TABLE 2

F_2 segregation of 14 W_2w_2 kafir heads.

PEDIGREE NUMBER	OBSERVED		*Calculated White*	DEVIATION	OBSERVED RATIO
	GREEN	WHITE			
3215-223-5-4-2	127	46	*43.25*	2.75±3.84	2.76:1
3	153	33	*46.50*	−13.50±3.98	4.64:1
4	133	58	*47.75*	10.25±4.04	2.29:1
6	116	33	*37.25*	− 4.25±3.57	3.51:1
7	107	36	*35.75*	.25±3.49	2.97:1
9	131	39	*42.50*	− 3.50±3.81	3.36:1
10	124	40	*41.00*	− 1.00±3.74	3.10:1
11	104	42	*36.50*	5.50±3.53	2.48:1
14	171	69	*60.00*	9.00±4.52	2.48:1
15	201	69	*67.50*	1.50±4.80	2.91:1
16	197	53	*62.50*	− 9.50±4.62	3.72:1
17	203	69	*68.00*	1.00±4.82	2.94:1
19	177	51	*57.00*	− 6.00±4.41	3.47:1
20	208	61	*67.25*	− 6.25±4.79	3.41:1
Total	2152	699	*712.75*	−13.75±15.59	3.08:1

The individual heterozygous heads and the total give very satisfactory 3:1 ratios and there seems to be no doubt that only a single factor is involved.

VIRESCENT SEEDLINGS

Genotype v_1v_1

This seedling chlorophyll deficient type was found in seed stock of Standard Blackhul kafir, Line 192, grown in 1923.

Virescent white seedlings in sorghum are at first practically without chlorophyll except for the tips of the first leaves which have a slightly greenish tinge. During the first few days after these first leaves unfold they turn to a very pale yellow and within a week or ten days fade out to almost white and die. There is considerable variation in the appearance of the virescent seedlings of a single progeny and also between the progenies from different parent heads. The chief difference is in the intensity of yellow. Various lots of V_1v_1 stock have been run through the greenhouse at different times of the year when the varying intensity of the light and heat was observed to affect the appearance of the recessive seedlings.

This virescent type of seedling in sorghum is similar to those which EMERSON (1912), MILES (1915), LINDSTROM (1918) and PHIPPS (1929) studied in maize and particularly the one described by DEMEREC (1924) as the v_2v_2 type of virescent maize seedlings.

Usually the v_1v_1 factor is lethal in the field but occasionally a few of the plants survive to maturity. Five out of thirty-four homozygous recessive plants survived in 1925 and produced seed. Two of them were very small and weak, but the others attained about three-fourths of normal development and seed production. Four of these were classed as green plants in the seedling stage, but when about two or three weeks old they developed yellowish-white striped areas in the leaves, the tissue lacking chlorophyll development fading out to white and later sloughing away. The progeny from these plants showed a wide range of variation in chlorophyll, some being almost completely white and others attaining almost normal chlorophyll development. Seventy-one percent of the seedlings which came up survived throughout the season. After sixty days nearly all of the plants were still very much retarded in development, varying in height from six inches to the height of normal plants. Only a few plants produced good heads and these were almost normal green at maturity.

TABLE 3

Segregation for virescent white seedlings in sorghum.

PEDIGREE NUMBER	NO. OF PROGENIES			NO. OF SEEDLINGS IN SEGREGATING HEADS				OBSERVED RATIO
	Homozygous green	Segregating		OBSERVED		*Calculated virescent*	Dev. ± P. E.	
		Actual	*Calculated*	Green	Virescent			
In 1925:								
192—8	40	73	75.3	4060	1201	1315.3	−114.3±21.2	3.38:1
In 1926:								
192—8— 3	24	58	54.7	3138	881	1004.8	−123.8±18.5	3.56:1
— 4	9	47	37.3	3538	925	1115.8	−190.8±19.5	3.82:1
— 5	18	46	42.7	4548	1120	1417.0	−297.0±22.0	4.06:1
— 6	13	39	34.7	4709	1265	1493.5	−228.5±22.6	3.72:1
— 7	34	55	59.3	5713	1607	1830.0	−223.0±25.0	3.56:1
— 8	43	69	74.7	5978	1704	1920.5	−216.5±25.6	3.51:1
— 9	30	67	64.7	5564	1440	1751.0	−311.0±24.4	3.86:1
—11	39	70	72.7	6329	1608	1984.3	−376.3±26.0	3.94:1
—12	15	20	23.3	1663	435	524.5	− 89.5±13.4	3.82:1
Total	265	544	*539.4*	45240	12186	*14356.7*	−2170.7±70.0	3.71:1
		Dev.=4.6±9.04					Dev./P.E.=31.01	
		Dev./P.E.=.51						

The original head produced 52 normal green and 15 virescent white seedlings. The original notes show that three of the 15 recessives were classed as albinos but later evidence indicates that they must have been virescents. Remnant seed of this head were planted and 114 F_2 progenies grown to maturity for further tests. Greenhouse tests of these progenies showed a consistent excess of green seedlings which could not be accounted for so that in 1926 nine heterozygous heads were planted and a progeny of from 50 to 100 selfed plants grown from each. The expected 2:1 ratio of homozygous green to heterozygous plants in these progenies was very satisfactory with a deviation of only 4.6 ± 9.04 plants. An even greater excess of green seedlings, however, was obtained from the 544 segregating heads with as careful a classification as could be made of more than fifty thousand seedlings. The deviation was 31.01 times its probable error and indicates clearly that it was not due merely to chance. The ratio was 3.71:1.

After becoming more familiar with the character and the normal variations common to the virescent white seedlings we concluded that some of them were being classed as normal green. The factor is usually lethal in effect but a few of the homozygous virescents survive. Progeny from such a plant of genetic constitution v_1v_1 range from almost completely lacking in chlorophyll development to almost normal green in appearance. The latter would almost certainly be classed as green in a segregating progeny.

In order to find whether or not the excess of greens in the 544 segregating progenies tested in 1926 were randomly distributed or due to a wide deviation from a 3:1 ratio in a few of the progenies, the deviations divided by the probable errors were classified as shown in table 4.

TABLE 4

Actual and theoretical distribution of Dev./P.E. for 544 heterozygous heads segregating 3:1 for green and virescent white.

	DEV. ÷P. E. CLASSES										
	.00–1.00	1.01–1.50	1.51–2.00	2.01–2.50	2.51–3.00	3.01–3.50	3.51–4.00	4.01–4.50	4.51–5.00	5.01–+	Total
Actual number	178.0	94.0	78.0	61.0	50.0	32.0	20.0	18.0	5.0	8.0	544
Theoretical number	*272.0*	*102.4*	*73.1*	*46.5*	*26.5*	*13.5*	*16.4*	*2.5*	*.9*	*.4*	*544*
Actual percent	32.7	50.0	64.3	75.6	84.7	90.6	94.3	97.6	98.5	100.0	100
Theoretical percent	*50.0*	*68.8*	*82.3*	*90.8*	*95.7*	*98.2*	*99.4*	*99.8*	*99.9*	*100.0*	*100*

Actual deviations of two or more times their probable error are considerably in excess of theoretical expectations. Eight of the progenies deviated from a 3:1 ratio by five or more times their probable error, which is twenty

times as many such deviations as would be expected if due to chance alone. The random distribution of the deviations and their consistency for all of the heterozygous heads tested is seen in figure 1.

The virescent white seedlings are clearly due to a single gene, but there are apparently modifying factors involved in the expression of the character that make the recessives difficult to classify accurately.

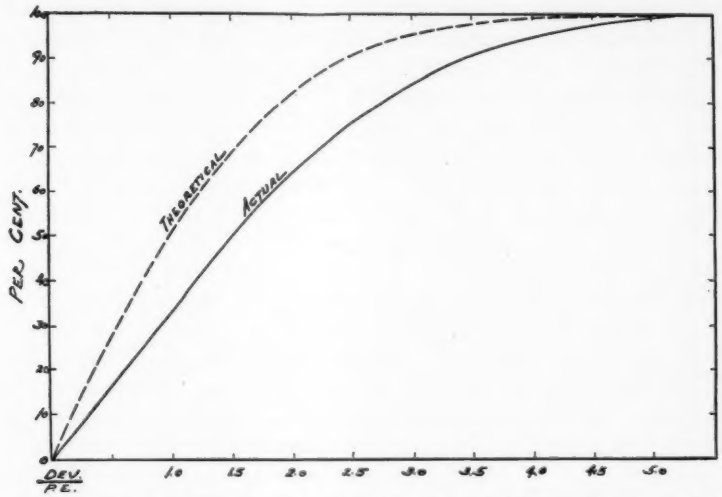

FIGURE 1.—Deviation from a 3:1 ratio in relation to the probable error for progeny of 544 heads segregating for green and virescent white. Actual and expected in cumulative percent.

Genotype v_2v_2

The origin of this chlorophyll character, which has been designated as virescent yellow, is of unusual interest. In 1924 a planting of 100 seed of a strain of Blackhul kafir, Line 153, inbred for seven generations, was sown in a flat. One of the seedlings was of a distinctly pale yellow to yellow color while the others were normal green. This single variation seemed unusual, and an additional 1000 seeds were planted from the same selfed head. Only the one abnormal seedling was found among the 1079 grown, indicating a chlorophyll character of complex inheritance or a mutation discovered soon after its inception. Later tests proved this character to be due to a single factor and it is possible that the particular seed producing this seedling was developed from the union of an ovule and pollen grain, both of which had

simultaneously mutated at the same loci or gene. Unfortunately, none of the normal seedlings from this same head were grown to maturity to determine whether they were heterozygous for this character. There is also the possibility that the mutation might have occurred but once in a primordial cell of this single floret.

In the early seedling stages the v_2 type of seedlings are readily distinguished from normal green, having a distinct pale yellow to yellow appearance. As development progresses the plants are apparently able to develop more chlorophyll and gradually turn to light green or pale green color at a

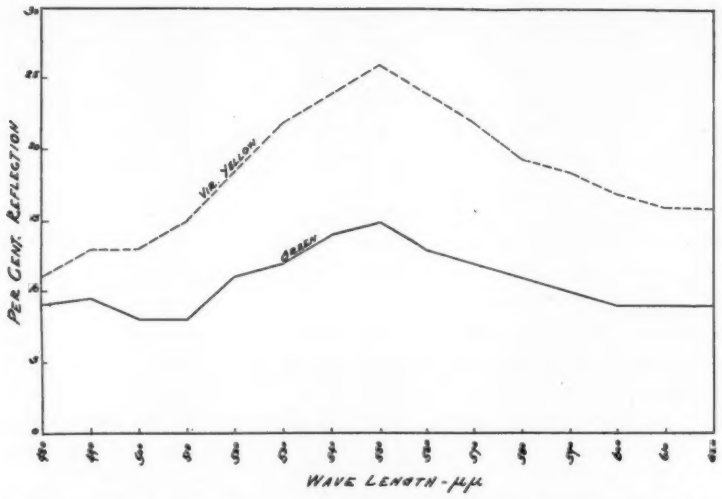

FIGURE 2.—Spectral reflection curves of virescent yellow and normal green sorghum leaves.

month to two months old and retain this color to maturity. Variation in color of individual plants is slight. Evidently there is not sufficient chlorophyll for normal photosynthesis as v_2 plants are materially retarded in their development. At six weeks homozygous virescent yellow plants measured 100 cm while the normal green plants were 130 cm tall. Full boot, heading, and maturity of the virescent plants are delayed fully two weeks later than normal plants.

A comparison of the color of homozygous virescent and normal green plants was obtained through the use of a spectrophotometer or color analyzer. Samples of leaves for making the color determinations were taken

from the same position on plants of the same age (8 weeks) and the spectral distribution of their stimuli measured and recorded. Readings were taken through the wave lengths 480 to 620 millimicrons, which is inclusive of the range of the stimuli of these colors. Spectrophotometric curves for the normal and virescent plants presented (figure 2) show an appreciable difference between their light reflecting properties as expressed in percentage of the reflection of standard white. These curves give a definite measure of the color of this chlorophyll abnormality in comparison with normal green sorghum.

An estimate of the chlorophyll content of the deficient type was made in comparison with normal plants. Extractions were made from leaves of both types with ethyl alcohol, carotin and xanthophyll separated out with petrol-ether, and chlorophyll estimated by matching the two solutions with a calorimeter. Virescent leaves at eight weeks were found to contain 90 percent as much chlorophyll as normal leaves. As seedlings they undoubtedly are much lower in chlorophyll content and even at maturity this pigment never fully develops.

The original seedling was potted off and grown to maturity. Later a number of crosses were made between this chlorophyll deficient type and normal green kafir plants. Seven F_1 plants were selfed and when tested in flats gave 2533 green and 767 virescent seedlings, or a ratio of 3.30:1. One of these segregating heads was planted and a selfed progeny of 136 heads produced in the F_2 generation. The segregation of these plants in the F_3 is given in table 5.

TABLE 5

Breeding behavior of the progeny of a plant heterozygous for virescent yellow seedlings.

GENOTYPE	NUMBER OF PROGENIES		NUMBER OF SEEDLINGS		*Calculated virescent yellow*	DEVIATION	RATIO
	Observed	*Calculated*	Green	Virescent yellow			
V_2v_2	89	*85.3*	10,312	3162	*3368.5*	206.5±33.93	3.26:1
V_2V_2	39	*42.7*	All				
v_2v_2	8	*42.7*					

In all of the segregating progenies there was a slight deficiency of the homozygous recessives due, probably, to differential viability. Considering the whole population from the segregating plants, a ratio of 3.26:1 is obtained and a single factor is undoubtedly responsible for this character. The deviation from the expected number, however, is significant and is

apparently due to lower viability of the recessives. Breeding behavior in the F_3 shows the number of V_2V_2 and V_2v_2 individuals to be close to the expected number when these two classes are considered alone, but only about one-fifth as many of the true breeding homozygous recessives were found as would be expected if all survived.

YELLOW SEEDLINGS

Genotype y_1y_1

Yellow seedlings have been found in a number of the common field crops. Their occurrence and inheritance has been reported in maize by LINDSTROM (1918, 1925), in barley by NILSSON-EHLE (1922), in cotton by STROMAN and MAHONEY (1925), and in wheat by HARRINGTON and SMITH (1928). In cotton and wheat two factors were involved in the inheritance of the character while in barley only a single gene was concerned. In maize two genetically distinct types of yellow seedlings were found.

TABLE 6

Segregation for yellow seedlings in F_3 Sudan grass progenies.

PEDIGREE NUMBER	OBSERVED		*Calculated Yellow*	DEVIATION	OBSERVED RATIO
	GREEN	YELLOW			
59—1—4	234	77	*77.75*	− 0.75±5.15	3.04:1
5	310	87	*99.25*	−12.25±5.82	3.56:1
7	216	62	*69.50*	− 7.50±4.87	3.48:1
10	385	145	*132.50*	12.50±6.72	2.66:1
11	359	93	*113.00*	−20.00±6.21	3.86:1
12	311	101	*103.00*	− 2.00±5.93	3.08:1
13	175	64	*59.75*	4.25±4.52	2.73:1
14	136	38	*43.50*	− 5.50±3.85	3.58:1
15	128	36	*41.00*	− 5.00±3.74	3.56:1
17	131	46	*44.25*	1.75±3.89	2.85:1
18	258	73	*82.75*	− 9.75±5.31	3.53:1
19	236	73	*77.25*	− 4.25±5.13	3.23:1
20	182	58	*60.00*	− 2.00±4.52	3.14:1
22	218	81	*74.75*	6.25±5.05	2.69:1
24	242	81	*80.75*	.25±5.25	2.99:1
25	161	59	*55.00*	4.00±4.33	2.73:1
26	217	71	*72.00*	− 1.00±4.96	3.06:1
Total	3899	1245	*1286.00*	−41.00±20.95	3.13:1

A chlorophyll deficient seedling type, designated as y_1y_1 was found in Sudan grass in 1924. The homozygous recessive is bright yellow in color and completely lethal in the seedling stage. Like albino seedlings they die

as soon as the food reserve in the seed becomes exhausted. Color in the recessives is usually quite constant and they are easily classified; however, the seedling progeny of Nos. 59-1-19 and 59-1-24 showed a variation toward a light yellow or virescent yellow which made them difficult to classify accurately after they were five to six days old.

The original plant of Sudan grass carrying the factor for yellow seedlings was selected at the Agronomy Farm at College Station. Preliminary trials with open fertilized seed gave ratios of 5.28:1 and 3.58:1 for yellow and green seedlings. A progeny of 26 selfed plants were grown in 1928 from remnant seed of a segregating plant. Nine of the plants proved to be genetically homozygous green and 17 segregated for green and yellow seedlings as shown in table 6.

A single factor is responsible for this yellow seedling recessive in sorghum. Natural cross-fertilization undoubtedly accounts for the excess of green seedlings found in the initial tests. There is a slight deficiency of the y_1y_1 class in many of the families but the deviation is, on the whole, less than twice its probable error. Total green and yellow seedlings in all families tested are in a ratio of 3.13:1.

Genotype y_2y_2

Another lethal yellow seedling character in sorghum was found in the progeny of a strain of inbred kafir, Line 192, which has previously, in this paper, been shown to be segregating for virescent white seedling. Seed from a single head of No. 192–35, which had previously segregated 3:1 for green and virescent seedlings, were planted in 1928 and a progeny of 29 plants harvested. All segregating plants produced green and virescent white seedlings except plant No. 15 which gave 207 green and 73 yellow seedlings, or a ratio of 2.84:1.

The recessive seedlings are uniform in color and brighter or deeper yellow than the genotype y_1y_1 described above. Homozygous recessives die in the seedling stage.

Eleven plants were grown in 1928 from the parent head carrying the y_2y_2 factor. Seed produced were immature, resulting in small seedling populations which were, however, adequate to show the nature of inheritance of the character. Data in table 7 show the seven heterozygous heads segregating green and yellow in a 3.11:1 ratio, indicating a single factor difference.

No virescent white seedlings appeared either in this parent head or among its progeny but were common to the parent stock from which y_2y_2 arose. Had the character already been present and inherited in a simple

manner it would readily have been detected. It appears, therefore, that y_2y_2 is the result of a gene mutation occurring while the stock was being grown and tested for another chlorophyll character.

TABLE 7

Behavior of 11 progenies of a Y_2y_2 kafir plant.

GENOTYPE	NUMBER OF PROGENIES		NUMBER OF SEEDLINGS		*Calculated Yellow*	DEVIATION	RATIO
	Observed	*Calculated*	Green	Yellow			
Y_2y_2	7	*7.3*	722	232	*238.5*	6.5±2.35	3.11:1
Y_2Y_2	4	*3.6*	All				

LINKAGE OF RED STEM COLOR AND ALBINISM

A red stem seedling of sorghum was found among progenies growing in the greenhouse in 1925. To provide further material for study of this genetic character the plant was potted off and grown to maturity. Only a small quantity of seed was obtained because of poor development of the plant but of the 60 seedlings produced from this seed there were 31 with red stem normal chlorophyll, 15 red stem albinos, 13 non-red stem normal chlorophyll and one non-red stem albino. Since the plant proved to be heterozygous for both stem color and white seedlings and the ratio obtained indicated a possible linkage between the two characters, further tests were made.

The original red stem seedling was thought to be kafir, but later evidence points to the fact that it was a hybrid between kafir and Sudan grass. Red stem families have also been found in Sudan grass, and as kafir seedlings normally have green stems, the character probably came into the cross from the Sudan grass parent. Marked hybrid vigor exhibited among the progeny of the next generation delayed maturity and prevented obtaining seed of many of the plants. Seed in others were poor and low germination resulted in small seedling populations and unsatisfactory ratios; however, families segregating for all four classes, red stem normal chlorophyll, red stem albino, non-red stem normal chlorophyll, and non-red stem albino were obtained. Also, families with normal chlorophyll segregating only for stem color and red stem families segregating green and albino were among the progenies.

Classification in the progeny is quite readily accomplished, both for stem color and chlorophyll development. Red stem seedlings have anthocyanin development well distributed throughout the coleoptile and this

character is dominant to the non-red stem or green stem alellomorph. White seedlings with anthocyanin in the coleoptile are readily distinguished from the pure albinos; however, both are weak and spindling with narrow seedling leaves and low viability.

A greater mortality among the albino seedlings results in a shortage for these two classes. Experience with other white seedlings in Sudan grass shows the albino factor, in the case of many of the seeds, to be an embryonic lethal. The recessives are simply too weak to germinate and consequently the albinos constitute only 20.3 percent of the total seedling population instead of 25 percent. Further evidence of the shortage of albinos is found in the fact that four of the heads which segregated only for red stem normal chlorophyll and red stem albinos gave 1669 normal and 447 albino seedlings, or only 21.1 percent albinos, about the same as found in the seven families heterozygous for both factors.

If independent inheritance only were involved, then the segregation for stem color among the albinos should be in a ratio of 3:1, but there are actually 36 percent non-red stem seedlings in the total of these two classes. However, six families heterozygous for the red stem factor did give 1523 red stem to 487 non-red, or very close to the expected one-fourth in the recessive class, indicating that the segregation is normal when the albino factor is not involved.[1]

TABLE 8

Seedling progenies from parent plants with the genotype WRwr showing segregation for stem color and white seedlings with linkage between R and w.

PLANT NUMBER	WITH RED STEM COLOR		WITH NON-RED STEM COLOR		CROSSOVER PERCENT
	Green	Albino	Green	Albino	
1926—1	31	15	13	1	25.88±1.93
—2	52	11	26	4	45.51±1.65
1928—1	215	73	103	20	42.27±1.70
—2	152	41	67	9	40.43±1.73
—3	78	21	30	5	43.35±1.68
—4	40	8	21	3	45.31±1.65
Observed	568	169	260	42	41.34±1.71
Calculated 9:3:3:1	*584.46*	*194.82*	*194.82*	*64.94*	
Adjusted	568	221	260	55	
Calculated with 41.34 percent C. O.	*598.92*	*228.75*	*228.75*	*47.14*	

[1] Since this paper was submitted for publication, a paper by REED (1930) has appeared which also shows that the red stem seedling character is a simple Mendelian dominant.

Representatives of each of the three genotypes were grown for further verification of the seedling progeny. Table 8 shows the segregation in families segregating for all four classes of seedlings; green seedlings with red stems, green seedlings with non-red stems, albinos with red stems, and the double recessive class, albinos with non-red stems. It will be seen that there is clearly a shortage in the albino classes due to failure of these seedlings to appear. If these two classes are adjusted for poor germination by assuming that they should contain one-fourth of the seedlings, the distribution is improved but still differs significantly from the expected numbers based on a 9:3:3:1 ratio, X^2 being equal to 34.3 and $P < 0.01$.

Crossover percentage, calculated on the observed totals by FISHER'S (1928) product method formula for two 3:1 ratios, and with the use of IMMER'S (1930) tables, gives a crossover value of 41.34 ± 1.71 percent. Distribution of the progenies of six families from selfed heads shows an expected variation in the individual crossover percentages. Recombinations appear more frequently in the *Wr* and *wR* classes, indicating a cross in the repulsion phase. Calculated on a basis of the two green chlorophyll classes only, and also the two albino classes only, values of 24 and 37 percent are obtained for the crossover. Since the product method is least disturbed by differential mortality of zygotes and considers all four classes, the crossover by this method is taken as the most likely figure.

When the theoretical numbers for each class are calculated on a basis of 41.34 percent crossing over, a fairly good fit is obtained with X^2 equal to 7.438 and $P > 0.05$.

The phenomenon of linkage, which has been found in many other species of plants and animals more extensively studied, has not heretofore been reported in sorghum. The evidence is clear that the factors for red stem and albinism here studied belong in the same linkage group.

OTHER CHLOROPHYLL DEFICIENCIES

In addition to chlorophyll characters, the inheritance of which has already been presented, others have been found in sorghum but information upon their inheritance is not yet fully established. Several of these will be briefly mentioned.

Selections from strain No. 2–15 of a Sudan grass stock, when grown in 1928, gave some rather peculiar ratios for normal green and albino seedlings and indicated that there might be as many as three factors responsible for the white seedlings. The homozygous recessives are without any green coloring whatever but anthocyanin is highly developed in both coleoptile and seedling leaves. They die in four to five days. Families segregat-

ing 3.05:1, 10.8:1, 17.2:1 and 61:1 were obtained and seed from each planted the following year from which bagged heads were secured for further testing. Segregation in the next generation resembled that of the parent families somewhat but the albinos were late in coming up and invariably weak. A high correlation existed between the number and vigor of seedlings in the row and the number of albinos. Germination was usually poor and no doubt there is a differential viability among the albino seedlings. Because of weakness and lack of viability it does not seem possible to determine and separate different factors if present in this stock. A single factor for albinism, acting partly as an embryonic lethal as well as a seedling lethal, does not, however, account completely for the shortage of albinos since one hundred seed samples of some families gave over 90 percent normal green seedlings. If a single gene only is concerned, there must also be a differential mortality or functioning of gametes.

Another chlorophyll type appearing as pale yellow or light yellow seedlings in Sudan grass Selection No. 58 has not been fully studied. The character does not act as a complete lethal since many of the recessive seedlings develop into mature plants, retaining the pale yellow color to maturity. The pure homozygous recessive form has been isolated and propagated. A single field count of the progeny from a heterozygous plant gave 689 normal and 212 pale yellow seedlings, or a 3.20:1 ratio, indicating that the character is likely a simple Mendelian recessive.

In addition to the chlorophyll characters inherited as simple Mendelian recessives a case of maternal inheritance of chlorophyll in sorghum has been found. The character is not transmitted through the pollen. Striped parental plants produce normal green, yellow, and striped green and yellow seedlings, depending upon the nature of the somatic tissue upon which the seed are borne. Details of the inheritance of this chlorophyll deficiency and some puzzling irregularities giving rise to other chlorophyll types in this same stock are receiving further study.

DISCUSSION

The occurrence and inheritance of the various chlorophyll characters in sorghum here reported suggest several other interesting and significant features. First there is a striking similarity in these characters, their frequency and mode of inheritance, with various forms found by many workers with maize. Each of the thirteen chlorophyll deficiencies in sorghums mentioned here have very similar, if not identical, forms found in maize, suggesting possible parallelism of such characters in the two species. Coat color in rodents and a number of genetic characters in the various

species of Drosophila have been shown to occur in a similarly corresponding series. The more closely related the species the more nearly identical one would expect to find such an homologous series of genetic characters common to both. Studies yet unpublished show all the common cultivated varieties of sorghum to have ten chromosomes in the haploid stage, or the same number as maize. While sorghum pollen applied to the silk of corn has not successfully effected fertilization, it has caused stimulation which has been found by P. C. MANGELSDORF to be merely a swelling of the nucellar tissue. Since the two species apparently cannot be crossed, it is not possible to determine whether the characters common to both are allelomorphic but it does seem that these two species of Gramineae may bear a closer relationship than has heretofore been thought to exist.

Furthermore, DEMEREC, HUTCHISON, and others have shown that chlorophyll mutations in maize are most apt to be those which hinder rather than enhance the development of this pigment and, therefore, that the mutations having the more pronounced detrimental effects would probably be of the most frequent occurrence. This was found to be the case and white seedlings were the most prevalent of the chlorophyll types in maize. Among the mutations induced by X-rays STADLER also found such types the most common. Similarly, among the chlorophyll mutations so far found in sorghum, white seedlings are the most frequently encountered. The inheritance of three such mutations is described here and two others are mentioned. A point of differences arises, however, in the manner of occurrence of the recessive types in the two species. In maize, a naturally cross-fertilized species, these recessives seem to be preserved in the heterozygous condition and mutations thus already present are readily brought to light when the stock is selfed. After these characters, which are present in the stock when inbreeding is started, are eliminated, however, new characters arising in the inbred lines through mutation appear to be rare. Sorghum is largely a self-fertilized species, and while the chlorophyll deficient types encountered are not so numerous as found when maize is selfed they are, nevertheless, of quite common occurrence and apparently are the result of frequent mutation. Lines or strains of sorghum inbred and under observation for many generations without showing any chlorophyll deficient types suddenly mutate to the recessive form and in several instances, while a stock was being grown and studied for one type of recessive, a gene mutation giving rise to another chlorophyll type has undoubtedly occurred. These characters in sorghum, therefore, are apparently not already present in the heterozygous condition, as a rule, since practically all of those now

being studied have arisen through recent mutation. This might suggest that the rate of mutation in sorghum is considerably higher than that in maize.

SUMMARY

1. The frequency and diversity of chlorophyll recessives recently found in sorghum suggest the existence of a parallelism with the many types found in maize.

2. White seedlings seem to be the most frequent type of chlorophyll mutation in sorghums. Those described are inherited as simple Mendelian recessives.

3. Two types of virescent seedlings, v_1 (virescent white) and v_2 (virescent yellow), both simple recessives, are reported.

4. Two types of yellow seedlings, one found in Sudan grass and one in kafir, of different phenotypic expression, are assigned different genetic factors, y_1 and y_2. Both are of simple inheritance and lethal in effect.

5. The factors R (red-stem) and w (abino) are linked with a crossing over of 41.34 percent. This is the first case of linkage reported in sorghum.

6. White seedlings in Sudan grass gave ratios indicating as many as three pairs of multiple factors involved, but if present, they could not be determined and separated because of weakness of the stock. An albino factor, acting as a zygotic and gametic lethal, is probably a better explanation of the peculiar ratios obtained. A virescent yellow Sudan grass, in which the homozygous recessive survives to maturity, is apparently due to a single gene.

7. A chlorophyll deficiency in sorghum not transmitted through the pollen but strictly maternal in inheritance produces normal, yellow, and striped green and yellow seedlings, depending upon the nature of somatic tissue upon which the seed are borne.

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CYTOLOGY AND GENETICS OF CROSSES BETWEEN FOURTEEN- AND SEVEN-CHROMOSOME SPECIES OF WHEAT

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Crosses between species of Triticum belonging to the emmer group (n = 14) and ones belonging to the *vulgare* group (n = 21) have been investigated extensively and profitably from the cytological standpoint, but those between emmers and einkorns (n = 7) have hitherto been studied only in the first generation. The very high degree of sterility of F_1 of the latter type has presumably made it difficult to secure a sufficient number of plants of later generations. Recently, however, a considerable amount of material has become available and is the subject of the present report.

REVIEW OF BEHAVIOR IN F_1

In order to interpret the results obtained with plants of later generations it will be necessary to refer to the cytology of F_1. In the hybrid between *T. turgidum* (n = 14) and *T. monococcum* (n = 7) SAX (1922) found that 7 chromosomes from *turgidum* paired with those of *monococcum* and that the other 7 were left unpaired. The latter failed to move into the central position and remained intact at the heterotypic division, but divided at the homotypic. In a cross between other varieties of the same two species THOMPSON (1926) found the behavior to be quite different. The number of conjugated pairs varied from 3 to 7, the commonest being 5. Consequently

the univalents varied from 7 to 15. Usually all of the latter and always the majority of them moved to the equator after the bivalents and divided at the first division, as do those of other wheat hybrids. At the homotypic division the number of lagging univalents was quite variable, depending on the amount of non-conjunction at the previous division and on the number of univalents which had failed to move into the plate. Consequently the number of chromosomes in individual pollen grains must have varied widely. Usually it would be 10 or 11. But if only 3 pairs had mated at the first division and if all univalents had reached the same pole at the second, it would be as low as 3 or as high as 18.

Since that account was published hybrids between *monococcum* and *dicoccum*, *durum* and *polonicum* have been examined and in all of them the behavior was found to be the same as in *turgidum*×*monococcum*. Similar behavior was observed also by KIHARA and NISHIYAMA (1928) in *dicoccum* ×*monococcum* and by AASE (1930) in *durum*×*monococcum* and *dicoccoides* ×*monococcum*. It must therefore be regarded as the usual type in F_1 hybrids between 14- and 7-chromosome wheats.

METHODS

Owing to a large and variable amount of non-conjunction in plants of later generations and difficulty in distinguishing all univalents from bivalents at the heterotypic metaphase, it was very desirable to have polar views of the heterotypic anaphase for determining the total (somatic) number as well as the amount of pairing. In smear preparations only a small fraction of the cells presented polar views, because the axis of division was the long axis of the cells, which naturally tended to lie on their sides. Consequently in many cases paraffin sections had to be used. When these were cut transversely to the long axis of the stamen most of the mother cells presented polar views, and when they were at anaphase counting of both groups of bivalents and the intervening univalents presented little difficulty. In all cases, however, smears were first made because they were excellent for determining the frequency of the different numbers of univalents, and if the amount of pairing was fairly constant and the total number not too large, complete determinations could usually be made from them alone. Part of the fixed material of each plant, preserved in 70 percent alcohol, was therefore first examined in smears, and in case the results were doubtful the rest was imbedded and sectioned.

The 7-chromosome species used was *T. monococcum* and the 14-chromosome species *T. durum* and *T. turgidum*.

FERTILITY

Using the average number of spikes per plant and of flowers per spike, and the total number of grains obtained, it is estimated roughly that about 1 F_1 flower in 800 or 900 formed a grain when the plants were grown unprotected in an ordinary plot near other wheat plants. Most of the grains were the product of self-pollination which is the rule in wheat, but some resulted from natural cross-pollination which was favored by the high degree of sterility. When the first lot of F_1 were grown, protection was neglected because of the habitual self-pollination of the parents. When the spikes were bagged the number of grains was somewhat reduced, but evidently gametes of both sexes were capable of functioning.

More than 98 percent of the F_1 pollen grains were shrunken and without contents.

Some results of artificial backcrossing are given in table 1. It appears that the F_1 cannot be backcrossed either as male or female with the 7-chromosome parent. With the 14-chromosome parent the female gametes

TABLE 1

Results of backcrossing.

BACKCROSS	FLOWERS POLLINATED	GRAINS FORMED
F_1 ♀ × *monococcum* ♂	390	0
monococcum ♀ × F_1 ♂	440	0
F_1 ♀ × *durum* ♂	365	3
durum ♀ × F_1 ♂	258	1

were more fertile than the male, but in both the fertility was very low. Consequently the labor involved was too great to secure, by artificial backpollination, a sufficient number of offspring for a comprehensive study. The F_1 themselves, however, can be produced in large numbers, and when grown well spaced in favorable conditions bear an immense number of flowers from which a reasonable number of grains may be secured.

CHROMOSOMES IN F_2

The somatic chromosome numbers of 41 F_2 plants are given in table 2. It may be observed that nearly all had 28 or more chromosomes, that is, as many as or more than the parental species with the larger number, and that the remainder had only 1 or 2 fewer. No plant with a number approaching that of *monococcum* ($2n = 14$) was found.

This result is quite different from that obtained by several investigators

TABLE 2

Somatic chromosome numbers in F_2.

CROSS	CHROMOSOME NUMBERS							
	26	27	28	29	30	35	36	42
durum (28)×*monococcum* (14)	1	2	11	6	2	2	..	2
turgidum (28)×*monococcum* (14)	..	1	6	2	1	3	2	..
Total	1	3	17	8	3	5	2	2

in crosses between 28- and 42-chromosome (2n) species. In those, segregates with various numbers between 28 and 42 were found. In some crosses of that kind, and perhaps in all, there were far more segregates with the lower number of the emmer parent than with the higher number of the *vulgare* parent (THOMPSON and HOLLINGSHEAD 1927).

In the pollen mother cells of the majority of F_2 of the present crosses

TABLE 3

Number of univalent chromosomes observed in pollen mother cells of some individual F_2 plants.

SOMATIC NUMBER	UNIVALENTS OBSERVED IN POLLEN MOTHER CELLS	
	COMMONEST NUMBER	LARGEST NUMBER
26	2	4
27	1	3
	3	5
28	0	1
	0	2
	0	4
	0	5
	2	2
	2	4
	2	8
29	1	1
	1	2
	1	3
	1	5
	3	3
	3	7
30	2	4
	2	10

there was considerable variation in the number of chromosomes which paired, and consequently in the number of lagging univalents. Table 3 shows the commonest number of univalents observed and also the largest number in some individual F_2, the somatic numbers of which are shown in the column at the left. Table 4 shows the frequency distribution of the

TABLE 4

Frequencies of different numbers of univalents in certain F_2.

SOMATIC NUMBER	UNIVALENTS													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
26	5	3	38	..	4	..	..	..	..	..	..	..	..	..
27	..	1	4	19	2	7	..	..	..	..	..	..	..	..
28	25	1	2	..	..	..	..	..	..	..	..	..	..	..
	18	5	15	..	..	..	..	..	..	..	..	..	..	..
	3	..	17	4	10	3	8	1	2	..	..	..	..	..
29	2	20	2	2	..	..	..	..	..	..	..	..	..	..
	12	25	6	16	3	4	..	..	..	..	..	..	..	..
	..	6	2	7	3	10	2	5	..	..	..	..	..	..
35	..	..	..	3	7	11	15	14	..	..	..	..	..	..
	..	..	..	..	1	2	6	13	1	4	..	2	..	..
36	..	..	..	..	..	..	2	11	16	8	1	2	1	1
42	21	11	10	5	8	1	3	1	..	..	..	..	..	..
	..	..	..	..	2	1	7	13	5	4	5	1	..	..

numbers of univalents seen in several plants. The number of univalents seen in any particular cell may not have been all there were in that cell, owing to their being obscured by bivalents at metaphase, or to their failure to move into the central region after the bivalents had divided. The only chromosomes recorded as univalents were those concerning which there could be no doubt.

The tables show that different plants with the same somatic number exhibited quite different amounts of non-conjunction and different degrees of variability in the amount of non-conjunction. Thus in some 28-chromosome plants 14 bivalents and no univalents were usually seen, as was to be expected, although individual mother cells sometimes showed 1 or a few univalents. In other plants with 28 somatic chromosomes there were usually 2 univalents but frequently more than 2, and in one case as many as 8

were seen. Similar conditions prevailed in plants with other somatic numbers, but in general these were still more irregular. In many cases the number of univalents in a single stamen was so variable that it was difficult or impossible to decide what the normal number of pairs should be.

This variability is evidently related to the similar phenomenon in F_1 where 2 pairs often failed to conjoin and 3 or 4 pairs sometimes failed. It is conceivable that non-conjunction occurred about equally in the different pairs, now involving one pair and now another. But the F_2 results indicate rather that it occurred in certain pairs much more frequently than in others. If an F_2 plant received an emmer and a *monococcum* chromosome from such a pair it would presumably show much non-conjunction in this pair; if it received both members of the pair from emmer or from *monococcum*, it would show little. There are probably other factors also, because in some cases where there were usually no univalents or only one, sometimes a large number were seen.

In some plants trivalent chromosomes appeared to be frequent, but as it is often difficult to distinguish them from bivalents in this material with the methods used, no attempt was made to estimate their frequency. Their occurrence accounts for such observations as pollen mother cells without univalents in a 29-chromosome plant, the extra chromosome presumably having become attached to one of the bivalents.

Of the 41 plants, 5 had 35 chromosomes, 2 had 36, and 2 had 42. These plants may have been produced in 2 ways: (1) F_1 gametes with 21 chromosomes, the sum of the numbers in the parents, may have been formed and joined with similar gametes to produce 42-chromosome plants, or with 14- and 15-chromosome gametes to produce 35- and 36-chromosome zygotes respectively. (2) Pure *vulgare* male gametes, as a result of natural cross-pollination, may have fertilized 14-, 15-, or 21-chromosome F_1 eggs to produce 35-, 36-, or 42-chromosome plants respectively.

Some of the plants with 35-chromosomes and one of those with 42 had some *vulgare*-like characters and must have arisen as a result of out-crossing with some *vulgare* type, unless the combination of emmer and *monococcum* genes could produce *vulgare* characters. But the characters of the remainder including one 42-chromosome plant were such that they could not have had a *vulgare* parent. Consequently a considerable number of 21-chromosome F_1 gametes must have functioned. On any interpretation 2 F_1 eggs with 21 chromosomes must have functioned in order to produce 42-chromosome plants. In this connection attention may also be called to the facts that nearly half the mother cells of one 42-chromosome plant showed no unmated univalents and that the great majority had less than 3. If it

had resulted from the fertilization of a 21-chromosome F_1 egg by *vulgare* pollen most of the mother cells would have shown at least 7 univalents, as is the case in the other 42-chromosome plant.

A hexaploid wheat plant and several pentaploids have therefore been produced by crossing a tetraploid with a diploid.

F_1 GAMETES WHICH FUNCTIONED

Ordinary gametes

The chromosome numbers of F_2 plants show that the great majority of F_1 gametes which functioned successfully must have had 14 chromosomes, the number in the gametes of the emmer parent, or more than 14. Those with 13 functioned rarely, those with 12 probably and with less than 12 certainly not at all in the material studied. In addition there were a few unexpected gametes with 21.

The theoretical frequencies of the F_1 gametes with the different chromosome numbers which are to be expected on the basis of the behavior at the meiotic divisions, are shown in table 5 for cases in which 7, 6, or 5 pairs

TABLE 5

Chromosome numbers in F_1 gametes.

PAIRING IN MOTHER CELLS	5	6	7	8	9	10	11	12	13	14	15	16
7II+ 7I	..	..	1	7	21	35	35	21	7	1	..	..
6II+ 9I	..	1	9	36	84	126	126	84	36	9	1	..
5II+11I	1	11	55	165	330	462	462	330	165	55	11	1

conjugate. Mother cells with 5 bivalents are commonest; ones with 4 or 3 occur. In the commonest type (5II+11I) only 55 gametes among 2048 would have 14, 11 would have 15, and 1 would have 16. Obviously the great majority of the gametes—those with numbers from 5 to 12—were unable to function successfully.

This result is quite different from that obtained in hybrids between 14(n) and 21-chromosome wheats (THOMPSON and CAMERON 1928, SAX 1928). In those, some gametes with each number between 14 and 21 did function successfully, although most of the male ones and some of the female with intermediate numbers were inviable or unsuccessful. Moreover, those which had only 14 and therefore entirely lacked the differential set of 7 were quite viable; in fact they were more successful than those with 21. In the emmer-*monococcum* hybrids, on the other hand, those which lacked

the differential set and consequently had only 7, were quite unsuccessful as shown by the fact that no F_2 with 14 somatic chromosomes or any number approaching 14 were found. Apparently combinations of emmer and *monococcum* chromosomes making up a set of 7 from those kinds which paired in F_1 were inviable unless an additional set of 7 was present. The failure of backcrossing with *monococcum* shows that F_1 gametes with 7 could not function even in association with healthy vigorous gametes which had the same number.

It is of course possible that gametes with fewer than 14 were actually capable of carrying on their function, but that the resulting zygotes perished. However the fact that more than 98 percent of the pollen was visibly abortive indicates that the sterility was gametic. Even many of those grains which appeared normal might be unable to function, particularly in view of the highly abnormal conditions in a stamen with so much bad pollen.

From the commonest type of mother cells (those with 5 bivalents and 11 univalents) only 55 gametes among 2048 would have 14 chromosomes. And it seems probable that most of these 55 would be unable to function. If we designate as AA′ and BB′ the 2 pairs which failed to conjoin, thereby giving 5II and 11I instead of 7II and 7I, it is probable that only those with 1A and 1B would be capable of functioning. If they had 2A's they must have lacked B's or 1 of the differential set of 7. At any rate, they would be more likely to function successfully than the others. There were only 4 such gametes among the 55 with 14 chromosomes. On the other hand, the occurrence of 28-chromosome F_2 in which 2 or more univalents were usually to be seen in the meiotic divisions may indicate that some other combinations could function.

If the mother cells had 6II and 9I, 9 of 512 gametes would have 14 and 1 would have 15 chromosomes. Only 2 of the 9 may be expected to have viable combinations. If the mother cells had 7II and 7I, 1 of 128 gametes would have 14 and none 15. The proportion of 14-chromosome gametes with each kind of chromosome represented once, which would be produced from the different kinds of mother cells, is as follows:

7II+ 7I.....1 in 128
6II+ 9I.....1 in 256
5II+11I.....1 in 512
4II+13I.....1 in 1024

If the theoretical fertility of F_1 be calculated by multiplying the proportion of 14-chromosome eggs by that of 14-chromosome pollen, a figure

is obtained much lower than that actually found. But this method would be unwarranted because, owing to abortion of the pollen, the 14-chromosome grains have little competition. Each flower contains only 1 egg and each stigma receives many grains, some of which may have 14.

Gametes with restituted nuclei

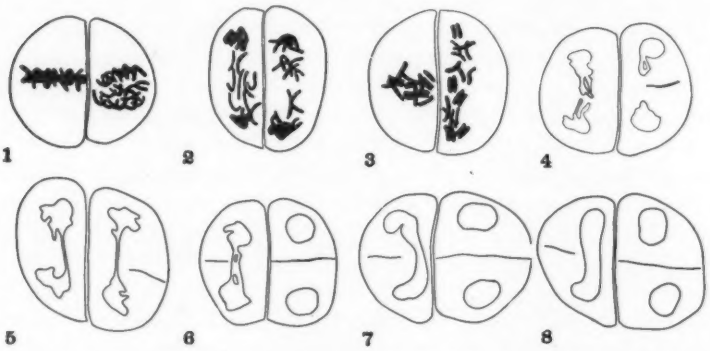
In order to determine whether some special or abnormal process in the behavior of F_1 chromosomes might be increasing the proportion of 14-chromosome gametes, the meiotic division of F_1 were carefully reexamined in material from many stamens. A second reason for this reëxamination was the desirability of explaining the occurrence of 21-chromosome gametes. That the latter were not due to doubling of chromosomes in early somatic tissues was evident from the fact that the grains occurred singly in ears which were otherwise sterile.

The heterotypic division showed nothing of significance in this connection. The lagging univalents, numbering from 7 to 15, divided in regular fashion; the halves separated cleanly and followed the bivalents to the poles in an orderly row. In almost every case all the chromosomes became incorporated in the daughter nuclei; very rarely were any lost or were micronuclei formed. No indication of a restitution of the somatic number was observed in many hundreds of cells at this division. Owing to the regularity of the division in spite of the large number of univalents, restitution would be very unlikely to occur. Therefore the interphase nuclei, which were always separated by a wall, would have about 14 chromosomes (7 halves of bivalents + 7 halves of univalents or 6+9, or 5+11). Occasionally the number would be somewhat smaller owing to 1 or a few univalents remaining undivided at one pole instead of moving into the plate.

The homotypic division, on the other hand, frequently presented significant appearances. It was usually very irregular. The halves of univalents remained for a long time in the central region, becoming much elongated, and moved very slowly and irregularly to the poles without dividing (figures 1 and 2). Usually the bivalents and the first univalents to arrive at the pole had formed the daughter nucleus while several univalents still remained outside; the latter then formed micronuclei. Partially incorporated univalents often connected the polar mass with one or more micronuclei. In many cases a series of univalents extended between the two daughter nuclei (figures 2, 4). Not uncommonly these formed a connecting bridge between the two nuclei at telophase and later. The bridge was usually slender (figure 5) but often nearly or quite as wide as the masses of chromatin at the ends (figures 6, 7). The chromosomes constituting the bridge

passed into the resting stage in the same way as those at the poles. In this manner a single nucleus, at first usually dumb-bell shaped, was sometimes reconstituted following the homotypic division (figures 7, 8).

The bridge varied in width from that of a single chromosome to a wide band composed of a considerable number. It is to be remembered that the number of univalents might be as high as 15, in which case the number of bivalents would be only 3. In such a cell the band of lagging univalents might be at least as wide as the groups of bivalents. Judging by the number of cells with restituted nuclei at different stages the slenderer bridges usually broke. The new wall commonly began to form across a portion only



FIGURES 1 to 8.—*T. durum*×*T. monococcum* F_1. (1) Metaphase and early anaphase of homotypic division. (2) Telophase bivalents, lagging univalents. (3) Chromosomes scattering without division. (4) Partially incorporated univalents. (5 and 6) Slender and stouter bridges formed from univalents. (7 and 8) Restituted nuclei. All figures are from smear preparations, were made with camera lucida, Zeiss apochromatic objective ×40, eyepiece×20, and were reduced to a magnification of about 1500.

of the diameter of the cell and later disappeared in cases of complete restitution. A proportion of the very young pollen grains were in triads instead of the usual tetrads.

As a result of the restitution gametes with 21 chromosomes (the sum of the numbers in the parental gametes) would be formed. If 7 pairs had conjugated in the prophases of the heterotypic division, the interphase nuclei would have 7 halves of bivalents and 7 halves of univalents, and the restituted pollen grain would have 7+7 from bivalents and 7 from univalents, making 21 in all. If 6 pairs had conjugated it would have 6+6+9=21; if 5 pairs, 5+5+11=21.

Several cases of the production of gametes with somatic chromosome number have been reported. These have resulted from the doubling of the chromosomes in somatic tissues or from a process of restitution following the heterotypic division as first described by ROSENBERG (1927). HÅKANSSON (1929) has recently reported the production of diploid gametes in Salix through an irregularity at the homotypic division, but his case apparently involves the fusion of the 2 homotypic spindles. In wheat this could not occur because a wall invariably separates the interphase nuclei. Our case is much more like that of ROSENBERG but restitution follows the homotypic division instead of the heterotypic as in his case.

Whether the restitution follows the heterotypic or the homotypic division seems to depend on the comparative degree of irregularity and in particular on the behavior of the univalents. Although these lag behind the bivalents in wheat, they divide in a very regular fashion at the first division, and move in an orderly line to join the bivalents, always arriving at the pole in time to become incorporated in the new nucleus. At the second division, on the other hand, since they fail to divide, they lag for a much longer time, move slowly and irregularly toward the ends, and become much elongated. All these conditions favor restitution of a single nucleus from the whole mass.

Although the number of chromosomes in the gametes would be the same when restitution follows the second as when it follows the first division, the constitution of the chromosomes might be quite different. For example, if 7 pairs had conjugated, restitution after the first division would always give 7 *monococcum* and 14 *durum* chromosomes, but after the homotypic it might give gametes with from 0 to 14 *monococcum* chromosomes, plus enough *durum* ones to make 21. If the interphase nucleus received the 7 *monococcum* halves of the bivalents, the restituted nucleus would have 2×7 *monococcum* chromosomes plus the 7 *durum* ones which never mate. If the interphase nucleus received no *monococcum* halves of the bivalents the restituted nucleus would have 2×7 *durum* chromosomes from bivalents, plus the 7 *durum* ones which never mate.

The only special process observed which tended to increase the proportion of 14-chromosome gametes was an occasional failure to form a plate at the second division. In these cases all the chromosomes failed to divide and simply scattered toward the poles (figure 3). Such cases were easy to distinguish from the usual anaphase by the number, size, and form of the chromosomes. At the usual type of division the chromosome plate extended almost completely across the cell (figure 1), whereas in cases of scattering the group was long and narrow. The scattering without division

was favored by the elongated form of many interphase nuclei. This form was apparently due to the incorporation of tardy univalents which had been near the outside of the cell at the heterotypic division and consequently were late and joined the edge of the daughter nucleus.

In such cases of scattering the conditions were favorable for restitution. But by the time it had occurred it was impossible to decide whether the division had been by scattering or of the usual type. If restitution should occur following scattering the new nucleus would have about 14 chromosomes (7 halves of bivalents plus 7 halves of univalents, or 6+9, etc.). In this way the proportion of gametes with approximately 14 chromosomes would be increased.

CHROMOSOMES IN F_3

Table 6 gives the chromosome numbers of the F_3 plants which have been examined. The single plant with 26 was the offspring of a 28-chromosome

TABLE 6

Somatic chromosome numbers in F_3.

F_2	F_3																
	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
28	1	..	11	..	..	..	..	..	..	..	..	..	..	..	..	..	..
29	..	1	5	3	..	..	..	..	..	1	..	..	..	..	..	..	..
30	..	..	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..
35	..	..	4	1	1	..	2	..	1	..	..	..	..	..	..	1	..
36	..	..	..	..	..	..	..	..	..	2	..	1	1	3	1	..	1
42	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3

F_2 in which at least 2 pairs usually failed to conjugate. The single 35-chromosome offspring of 29-chromosome F_2 was probably the result of natural cross-pollination by *vulgare*.

In general the chromosome behavior in F_3 was much more regular than in F_2. For example, in each of the 11 plants with 28 chromosomes which were the offspring of F_2 with the same number, the mother cells usually showed no univalents; in some of them none were ever seen; in others a few were occasionally observed.

All but 1 of the offspring of 35-chromosome plants had a smaller number,

and in future generations would apparently be reduced to the 28-chromosome condition. The offspring of 36-chromosome plants, on the other hand tended toward the 42-chromosome condition. The 3 offspring of 42-chromosome F_2 all had 42 chromosomes and showed only occasional non-con junction in 1 or 2 pairs.

EXTERNAL CHARACTERS

Owing to the small number of plants, the very large amount of pollen abortion, and the occurrence of some out-crossing, little would be gained by an attempt to analyze the inheritance of the individual characters. Nevertheless, in view of the chromosome situation it appears desirable to record the general genetic results.

The condition of the more prominent contrasting characters in the parental species and in F_1 is shown in table 7. The figures given in connection

TABLE 7

CHARACTER	*durum*	*monococcum*	F_1
Stem	Solid	Hollow	Solid
Spike density	Dense (3.0)	Very dense (2.0)	Near *durum*
Spikelet form	Narrow (1.5)	Very narrow (2.3)	Near *monococcum*
Empty glume			
Second tooth	Small	Prominent	Near *monococcum*
Rib	Absent or slight	Prominent	Intermediate
Ratio flowering glume to empty glume	¼ or less longer	¼ or more longer	Near *monococcum*
Palea	Not split	Split	Not split
Rachis			
Toughness	Tough	Very brittle	Brittle
Width	Narrow	Wide	Intermediate
Hairs	None below or few	Full length	Near *durum*
Basal hairs	Medium tuft	Large tuft	Medium tuft
Grain			
Flatness	Slight	Pronounced	Slight
Furrow	Deep	Slight	Near *durum*

with spike density are averages obtained by dividing the length of the spike in millimeters by the number of spikelets; those in connection with

spikelet form by dividing its length by its breadth. In regard to 7 of the 13 pairs of characters the *durum* condition is partially or completely dominant; in regard to 3 the *monococcum* condition is partially dominant. *Turgidum* is like *durum* in regard to most of these characters, although its contrast with *monococcum* is less in regard to some and greater in regard to others.

The *monococcum* condition of every character except density of spike was recovered among 50 F_2 *durum*×*monococcum*, including those studied cytologically. The hollow stem and indistinct furrow on the grain characteristic of *monococcum* appeared in about 1/15 of the plants (excluding out-crosses with *vulgare*); the long, narrow spikelets, split palea, extremely flat grains, prominent rib on empty glume, and large tuft of hairs at the base of the spikelet in from 1/5 to 1/8; the prominent secondary tooth of the empty glume, the brittle and the broad rachis as well as the full fringe of hairs on the rachis, in 1/3 to 1/5; the long flowering glume in nearly 1/2.

In view of the facts that only 4 of the plants examined cytologically had fewer chromosomes than the *durum* parent, that those 4 had only 1 or 2 fewer, and that the *durum* condition of the majority of characters was partially dominant in F_1, the large proportion of plants with individual *monococcum* characters is important. At the most only half the chromosomes could be from *monococcum*, usually only one-quarter, and often less. Seven of the 14 pairs present in most F_2 could include no *monococcum* chromosome. These considerations indicated that the distinctively emmer characters must be carried chiefly in the 7 chromosomes which pair with those of *monococcum*, whereas from emmer-*vulgare* crosses it has been concluded that the distinctively *vulgare* characters are carried in the extra 7 chromosomes which do not pair with those of emmers.

In crosses between emmer and *vulgare* the contrasting characters are associated in a body, nearly all being either present or absent in individual F_2. In our emmer-*monococcum* hybrids that was not the case. Many different combinations of *durum* and *monococcum* characters occurred.

A striking feature of the F_2 was the appearance of conditions not present in either parent and not intermediate between the conditions of the parents. For example, several plants had no beards. That they could not have been natural crosses with *vulgare* was shown by their other characters as well as by their chromosome numbers; and there were no beardless plants, other than *vulgare* types with which the F_1 might have crossed. Possibly the genes for beards in the two parents are in different chromosomes which do not mate with each other; in that case an F_2 plant might lack genes for beards entirely. Or an inhibiting factor which had been suppressed in one

of the parents might have been freed from its suppressor. Other examples of such new conditions were a secondary tooth of the empty glume more pronounced even than in *monococcum*, and the entire absence of rachis hairs and of the basal tuft.

SUMMARY

Crosses between emmer wheats (n = 14) and *monococcum* (n = 7) have been studied cytologically in the second and third generations.

In F_2 no plant had fewer than 26 somatic chromosomes; the great majority had from 27 to 30, approximately the number in one parent; a few had 35 and 42. There was much non-conjunction; the amount varied widely in the mother cells of the same plant and expecially from plant to plant.

F_1 gametes with fewer than 13 chromosomes did not function. The successful gametes had approximately 14 or 21.

Restitution of a single nucleus following the homotypic division sometimes occurred. This was due to the presence of a wide bridge formed from lagging univalents and connecting the groups at the poles. Restituted nuclei had 21 chromosomes, the sum of the gametic numbers of the parents. The proportion of 14-chromosome gametes may have been increased by restitution following homotypic divisions in which the chromosomes scattered without dividing.

A hexaploid wheat has been produced by crossing a tetraploid and a diploid. Its characters are not those of common hexaploids.

In spite of the absence of segregates with chromosome numbers approaching that of *monococcum*, each *monococcum* character appeared in a considerable proportion of F_2. The emmer characters are carried chiefly in the 7 chromosomes which pair with those of *monococcum*.

The material for this investigation was grown and fixed at the UNIVERSITY OF SASKATCHEWAN, Canada. The cytological examinations were made chiefly at the Division of Genetics, UNIVERSITY OF CALIFORNIA. I am greatly indebted to Professor E. B. BABCOCK for the use of the facilities of his Division, and to the NATIONAL RESEARCH COUNCIL of Canada for financial assistance.

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THE INHERITANCE OF CERTAIN CHARACTERS IN OENOTHERA HYBRIDS OF DIFFERENT CHROMOSOME CONFIGURATIONS[1]

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INTRODUCTION

Perhaps the most significant development in the genetics of Oenothera in recent years is the finding of constant differences in the linkage relationships of certain characters and the association of these variations with constant differences in the chromosome configurations of the plants concerned. RENNER (1927) has shown, for example, that in one cross a character may be closely or completely linked to a second character and independent of a third character, while in another cross the first character will be independent of the second and linked to the third. CLELAND and OEHLKERS (OEHLKERS 1926, CLELAND and OEHLKERS 1929) have found that characters which are independently inherited in hybrids with pairing chromosomes are linked in hybrids in which the chromosomes are associated in large rings during meiosis.

A similar relationship between linkage of characters and association of chromosomes is found in the crosses reported in this paper.

[1] Paper from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 344.

DESCRIPTION OF TYPES

Oenothera franciscana

Oenothera franciscana Bartlett has been grown extensively by Professor DAVIS who has described this species in some detail in his paper of 1916. The characters of *franciscana* that are important in the experiments herein reported are its red bud-cones (figure 1), yellow flowers, and the *franciscana* growth-habit. CLELAND (1922) found that *franciscana* had five chromosome pairs and a circle of four chromosomes. Since then KULKARNI (1928) has reported the breaking of the circle into two pairs preceding the heterotypic metaphase. The *franciscana* plants used in the following experiments were not examined cytologically, but there is evidence (reported below) that one parent plant had the circle of four chromosomes. *Oe. franciscana* appears to be genetically homozygous and is apparently free from lethals. There are almost no empty or shrivelled pollen grains.

"Franciscana sulfurea"

The hybrid called *"franciscana sulfurea"*[2] arose from the cross *Oe. biennis*×*franciscana* (DAVIS 1916). It agrees closely with *franciscana* in growth habit, but has green bud-cones (figure 1) and sulfur-colored flowers.

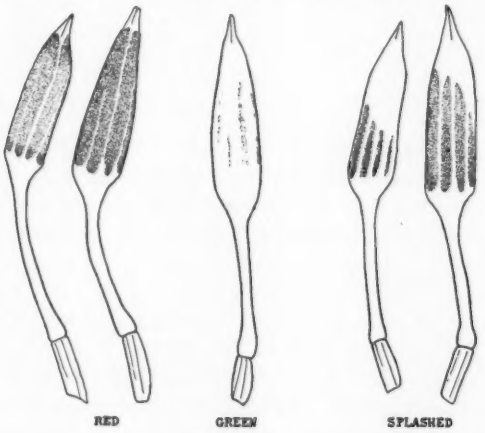


FIGURE 1.

[2] This form has also been grown by SHULL (1923b) who obtained his material from Professor DAVIS, but Professor SHULL's present cultures must represent a secondary derivative of the parent form as his plants have all pairing chromosomes (CLELAND 1927, ILLICK 1929) and apparently have red bud-cones—"similar to *franciscana* genetically except for its sulfur-colored flowers" (ILLICK 1929, p. 614).

Fifty percent of the pollen grains are empty and shrivelled. CLELAND (1924) found a circle of twelve chromosomes and a single free pair in this form. The parents used in the following experiments were not examined cytologically, but the cytology of F_1 culture 288 (reported below) of the cross with *franciscana* and of F_1 cultures of crosses with *Oe. Hookeri* suggested that the chromosome configuration of "*franciscana sulfurea*" had become altered. One plant (650-3), a descendant of the inbred parent of culture 288, happened to be still in flower in our garden in December. This plant was examined by the aceto-carmine method and found to have two pairs of chromosomes and apparently a circle of ten (plate 1).[3]

When inbred, "*franciscana sulfurea*" regularly produces the parent type and a new form called sulfur-dwarf.

Sulfur-dwarf

The sulfur-dwarf segregate from "*franciscana sulfurea*" differs from the parent form in bud color and in stature. It has red bud-cones similar to those of *franciscana*. In growth-habit it differs from *franciscana* chiefly in height. The central axis of the dwarf is roughly three-quarters as long as in *franciscana*. The lateral branches are generally fewer and less erect and the leaves somewhat narrower in the dwarf than in *franciscana*. Sulfur-dwarf has been found to have almost no empty pollen grains, and all of the chromosomes pair regularly (EMERSON 1928). Sulfur-dwarf breeds true.

Splashed bud-cones

A new bud color sometimes appears in crosses between *Oe. franciscana* and "*franciscana sulfurea.*" The bud-cones are distinctly red, but generally less pigmented than the red bud-cones of *franciscana*. The greatest difference is in the pattern of the pigmented area. Red bud-cones are strongly pigmented near the tip, while splashed bud-cones are always green near the tip (compare figure 1).

CROSSES BETWEEN "*FRANCISCANA SULFUREA*" AND SULFUR-DWARF

From self-pollinated "*franciscana sulfurea,*" a culture of 93 plants was grown (culture 394 of 1928), in which were 90 "*franciscana sulfurea*" plants and 3 sulfur-dwarfs.[4] The same plant pollinated by sulfur-dwarf yielded

[3] The inbred descendants of 650–3 have since been examined and found definitely to have a ring of 10 chromosomes and 2 pairs.

[4] Professor DAVIS has extensive unpublished data on the genetics of "*franciscana sulfurea.*" He has often found much higher percentages of sulfur-dwarfs in progenies of selfed "*franciscana sulfurea.*"

132 "*franciscana sulfurea*" plants and 9 sulfur-dwarfs (culture 397). In the reciprocal cross (culture 398) were 64 sulfur-dwarfs and no plants of the "*franciscana sulfurea*" type.

CROSSES BETWEEN "*FRANCISCANA SULFUREA*" AND *OENOTHERA FRANCISCANA*

Reciprocal crosses between *Oenothera franciscana* and "*franciscana sulfurea*" were made in the summer of 1923, from which F_1 cultures 58 and 59 were grown the following summer. In 1927, Professor DAVIS kindly gave me seeds of similar crosses made by him in 1925. From these seeds F_1 cul-

TABLE 1

F_1 frequencies in reciprocal crosses between Oenothera franciscana and "franciscana sulfurea."

CROSS	CULTURE NUMBER	BUD-CONE COLOR		
		RED	GREEN	SPLASHED
franciscana×"*franciscana sulfurea*"	58	76	0	0
	287	461	0	0
"*franciscana sulfurea*"×*franciscana*	59	14	45	1?
	288	255	203	1?

TABLE 2

Phenotypic frequencies in F_2 generations from F_1 plants with red bud-cones.

CULTURE NUMBER	YELLOW		SULFUR		PERCENT* GERMINATION	PARENT PLANT	CHROMOSOME† CONFIGURATION
	TALL	DWARF	TALL	DWARF			
177	55	1	3	0	?	58–1	⊙ 4, 5 pairs
178	67	5	14	2	?	59–1	7 pairs
406	26	15	0	0	68	287–3	7 pairs
411	45	3	8	0	59	−4	Unknown
416	16	2	1	0	33	−6	7 pairs
456	45	15	7	1	91	288–1	7 pairs
461	45	7	2	0	72	−2	Unknown
466	51	8	4	0	85	−4	7 pairs
470	47	4	1	0	70	−10	Unknown
473	45	7	1	1	75	−49	Unknown
Total	442	67	41	4			

* The germination percentages in this and following tables were observed from earth-sown seeds.

† In this and the following tables, the symbol ⊙ is used to indicate a chromosome circle. The number following the symbol indicates the number of chromosomes in the circle.

tures 287 and 288 were grown. The pedigrees of F_2, backcross, and F_3 generations are given in the following tables (1 to 14) in which the data obtained from these crosses are presented.

TABLE 3

Phenotypic frequencies in the backcross F_1 (red bud-cones)×sulfur-dwarf.

CULTURE NUMBER	YELLOW		SULFUR		PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	TALL	DWARF	TALL	DWARF			
409	29	28	6	7	97	287– 3	7 pairs
414	11	15	14	15	73	– 4	Unknown
417	30	29	5	7	95	– 6	7 pairs
420	34	21	11	4	97	– 10	7 pairs
425	19	14	20	21	96	– 31	7 pairs
427	18	18	4	2	96	– 40	Unknown
428	22	19	10	14	92	– 51	Unknown
429	29	18	7	9	91	– 90	Unknown
430	18	20	10	9	97	–110	Unknown
431	20	20	7	5	75	–140	Unknown
432	30	27	7	6	98	–165	Unknown
433	31	33	3	2	96	–200	Unknown
457	25	17	5	8	83	288– 1	7 pairs
464	2	1	0	2	57	– 2	Unknown
467	26	22	14	7	93	– 4	7 pairs
471	39	18	7	4	92	– 10	Unknown
474	16	23	5	8	69	– 49	Unknown
488	13	9	7	7	52	–103	Unknown
489	16	13	5	3	53	–106	Unknown
Total	428	365	147	140			

TABLE 4

Phenotypic frequencies in the backcross sulfur-dwarf×F_1 (red bud-cones).

CULTURE NUMBER	YELLOW		SULFUR		PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	TALL	DWARF	TALL	DWARF			
410	17	10	18	5	72	287– 3	7 pairs
415	8	6	1	1	86	– 4	Unknown
418	12	15	10	15	72	– 6	7 pairs
422	8	15	8	5	48	–10	7 pairs
424	12	23	9	9	72	–19	Unknown
458	10	12	3	3	37	288– 1	7 pairs
465	12	10	7	0	73	– 2	Unknown
472	3	2	2	2	21	–10	Unknown
475	2	3	2	3	16	–49	Unknown
Total	84	96	60	43			

TABLE 5

Phenotypic frequencies in the backcross F_1 (red bud-cones)×red sulfur tall.

CULTURE NUMBER	YELLOW	SULFUR	PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
407	52	12	88	287– 3	7 pairs
412	41	18	83	– 4	Unknown
419	66	12	68	– 6	7 pairs
434	37	19	77	– 7	7 pairs
435	55	4	91	– 8	Unknown
436	49	18	92	– 9	Unknown
437	52	22	100	– 12	Unknown
438	43	23	92	– 13	Unknown
440	34	16	67	– 33	Unknown
441	22	13	47	– 34	Unknown
442	24	15	53	– 36	Unknown
443	26	15	61	– 37	Unknown
444	10	5	20	– 38	Unknown
445	36	17	71	–201	Unknown
446	20	8	37	–202	Unknown
447	38	11	67	–203	Unknown
448	12	6	24	–204	Unknown
449	31	18	67	–205	Unknown
450	34	11	61	–206	Unknown
451	27	19	63	–207	Unknown
454	30	3	79	–211	Unknown
455	20	10	44	–212	Unknown
459	52	9	84	288– 1	7 pairs
462	56	6	83	– 2	Unknown
491	48	18	66	– 81	Unknown
476	53	31	59	– 89	Unknown
477	19	10	?	– 97	Unknown
490	35	24	61	–142	Unknown
478	9	4	17	–146	Unknown
479	34	17	68	–152	Unknown
480	28	19	65	–153	Unknown
481	59	5	73	–156	Unknown
482	27	12	41	–193	Unknown
483	43	23	69	–203	Unknown
484	20	4	32	–220	Unknown
485	40	24	87	–240	Unknown
486	33	25	81	–302	Unknown
487	25	8	44	–367	Unknown
Total	1340	534			

TABLE 6

Phenotypic frequencies in the backcross red sulfur tall×F_1 (red bud-cones).

CULTURE NUMBER	YELLOW	SULFUR	PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
408	44	20	92	287–3	7 pairs
413	32	17	?	–4	Unknown
460	40	23	87	288–1	7 pairs
463	23	5	65	–2	Unknown
469	51	12	87	–4	7 pairs
Total	190	77			

TABLE 7

Phenotypic frequencies in F_2 generations from F_1 (red bud-cones) F_2 red yellow tall.

CULTURE NUMBER	YELLOW		SULFUR		PARENT PLANT	CHROMOSOME CONFIGURATION
	TALL	DWARF	TALL	DWARF		
306	53	..	2	..	178– 3	Unknown
307	108	..	..	..	– 7	Unknown
308	21	0	1	1	– 9	Unknown
309	71	..	..	..	–10	Unknown
311	47	..	4	..	–16	Unknown
324	21	..	..	..	177– 1	Unknown
325	63	..	..	..	– 3	7 pairs
326	51	..	..	..	– 9	⊙ 4, 5 pairs
329	8	..	..	..	–24	⊙ 4, 5 pairs
330	66	..	..	..	–29	Unknown
331	34	..	18	..	–32	Unknown
332	43	13	..	..	–36	Unknown
333	25	..	4	..	–39	Unknown

TABLE 8

Phenotypic frequencies in F_2 generations from F_1 (red bud-cones), F_2 red sulfur tall.

CULTURE NUMBER	TALL	DWARF	PARENT PLANT
334	88	..	177–61
335	49	16	–59
336	55	19	–18
317	10	..	–83
319	15	..	–69

TABLE 9

Phenotypic frequencies in F_2 generations from F_1 plants with green bud-cones.

CULTURE	GREEN		SPLASHED YELLOW	RED		GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	YELLOW	SULFUR		YELLOW	SULFUR			
180	20	1	1	24	0	?	59– 3	Unknown
494	23	0	7	43	0	74	288–40	Unknown
496	43	0	2	64	1	100	– 5	⊙ 10, 2 pairs
501	17	0	2	29	0	48	– 6	Unknown
505	18	0	1	29	0	50	– 7	Unknown
525	20	0	3	25	0	49	–20	⊙ 10, 2 pairs
531	1	0	0	23	0	28	–29	Unknown
534	0	0	0	3	0	4	-60	2 pairs, 1 trivalent, chain 7
Total	142	1	16	240	1			

CHROMOSOME CONFIGURATIONS OF THE HYBRIDS

Cytological examinations were made of two red-budded plants of the F_1 generations grown in 1924. Of these, one plant (58–1) had a circle of four chromosomes and five chromosome pairs, the other plant (59–1) had seven chromosome pairs. In an F_2 culture (177) from 58–1, three plants were examined. Two of these had circles of four chromosome and five pairs, the third had seven chromosome pairs.

From F_1 cultures grown in 1927, ten plants with red bud-cones were examined. One plant (287–1) proved to be a haploid (EMERSON 1929). The other plants each had seven pairs of chromosomes (plants 287–3, –5, –7, –10, –31, 288–1, –4, and –81).

Four plants with green bud-cones in the F_1 cultures of 1927 were examined cytologically. Of these, three plants had each a circle of ten chromosomes and two pairs (288–5, –20, –71), while one plant (288–60) had two chromosome pairs, a trivalent group, and a chain of seven (or eight) chromosomes. Only a few good figures were obtained from plant 288–60, but in each telophase examined there were seven chromosomes in each daughter nucleus. It will be noted in table 9 that self-pollinated seeds of plant 288–60 germinated very poorly. A recent test using about two hundred seeds of this plant gave no germination while seeds of a sister plant still germinated perfectly.

No preparations were obtained from plants with green bud-cones of F_1 culture 59.

The chromosome configurations of the various plants listed above are illustrated in plates 1 to 3.

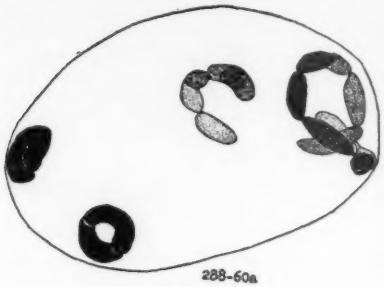
650-3a
650-3b
288-5a
288-5b
288-71a
288-71b
288-20
0 1 2 3 4 5
micra

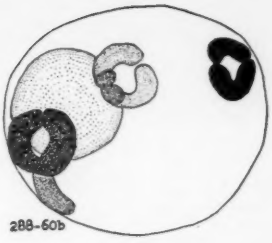
TABLE 10

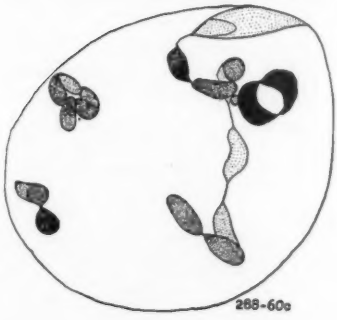
Phenotypic frequencies in the backcross F_1 (green bud-cones) × red sulfur.

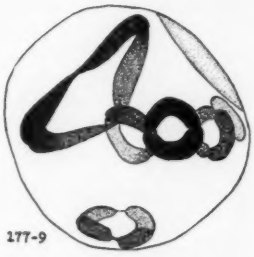
CULTURE	GREEN		SPLASHED		RED		GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
	YELLOW	SULFUR	YELLOW	SULFUR	YELLOW	SULFUR			
495*	1	1	2	0	28	1	52	288– 40	Unknown
497	3	20	1	0	48	3	77	– 5	⊙ 10, 2 pairs
499*	0	18	2	0	52	3	76		
502	0	5	1	0	26	0	64	– 6	Unknown
506	1	15	1	1	47	0	69	– 7	Unknown
508*	3	20	0	1	49	4	82		
509	0	3	1	0	17	1	24	– 71	⊙ 10, 2 pairs
510	0	14	3	0	34	2	57	– 82	Unknown
511	0	6	2	1	35	0	45	– 83	Unknown
512	0	6	6	0	20	0	34	– 84	Unknown
513	0	4	2	0	23	1	34	– 91	Unknown
514	1	22	0	0	33	3	62	– 92	Unknown
515	1	17	0	1	50	0	69	– 93	Unknown
516	0	5	1	0	35	1	43	– 94	Unknown
517	0	8	0	0	16	2	27	– 99	Unknown
518*	0	11	2	0	20	1	34	–101	Unknown
519	0	6	1	0	13	0	23	–107	Unknown
520	3	6	0	0	18	1	29	–112	Unknown
521	0	2	1	0	35	2	43	–120	Unknown
522	3	7	0	0	18	1	30	–130	Unknown
523	1	9	0	0	28	2	39	–140	Unknown
526	0	2	0	0	4	0	6	– 85	Unknown
527	0	6	1	0	20	1	28	– 86	Unknown
528	0	6	1	0	26	3	37	– 87	Unknown
529*	0	6	1	0	31	1	39	– 20	⊙ 10, 2 pairs
532*	1	3	0	0	28	0	33	– 29	Unknown
535	0	4	0	0	7	0	11	– 60	2 pairs, 1 trivalent, chain 7
536	2	9	0	1	58	1	76	–319	Unknown
537	0	12	0	0	21	0	35	–356	Unknown
538	0	2	0	0	16	1	21	–143	Unknown
539	0	0	0	0	7	1	8	–144	Unknown
540	1	9	1	0	29	3	49	–154	Unknown
541	6	7	0	0	33	1	50	–259	Unknown
542	0	3	1	0	23	1	30	–286	Unknown
Total	27	274	31	5	948	41			

* A culture which was backcross to sulfur-dwarf. Since there were no dwarf segregates from hybrids with green bud-cones, these cultures are listed with the backcrosses to tall red sulfur.


288-60a


288-60b


288-60c


177-9

58-1

177-24

287-6

287-31

TABLE 11

Phenotypic frequencies in the backcross red sulfur×F_1 (green bud-cones).

CULTURE NUMBER	SPLASHED YELLOW	RED		PERCENT GERMINATION	PARENT PLANT	CHROMOSOME CONFIGURATION
		YELLOW	SULFUR			
498	5?	41	0	61	288– 5	⊙ 10, 2 pairs
500*	0	1	0	2		
503	1	39	0	91	– 6	Unknown
504*	1?	57	3	80		
507	2	38	1	42	– 7	Unknown
524*	0	65	0	66		
530*	2	17	0	58	–20	⊙ 10, 2 pairs
533*	0	39	0	40	–29	Unknown
Total	5+6?	297	4			

* A culture which was a backcross to sulfur-dwarf. Since there were no dwarf segregates from hybrids with green bud-cones, these cultures are listed with the backcrosses to tall red sulfur.

TABLE 12

Phenotypic frequencies in F_2 generations from F_1 (green bud-cones) F_2 green yellow.

CULTURE NUMBER	GREEN		SPLASHED YELLOW	RED YELLOW	PARENT PLANT
	YELLOW	SULFUR			
289	19	0	0	17	180–20
290	16	0	0	15	–25
291	20	1	2	10	–29
292	9	0	1	11	–37
293	16	1	0	17	–39
294	18	0	1?	15	–47
295	4	0	1	24	–53
Total	102	2	4+1?	109	

TABLE 13

Phenotypic frequencies in F_2 generations from F_1 (green bud-cones)F_2 red yellow.

CULTURE NUMBER	RED YELLOW	PARENT PLANT
297	125	180– 2
298	71	– 5
299	102	– 8
300	99	–12
301	14	–15
302	110	–16
303	111	–17
305	110	–52

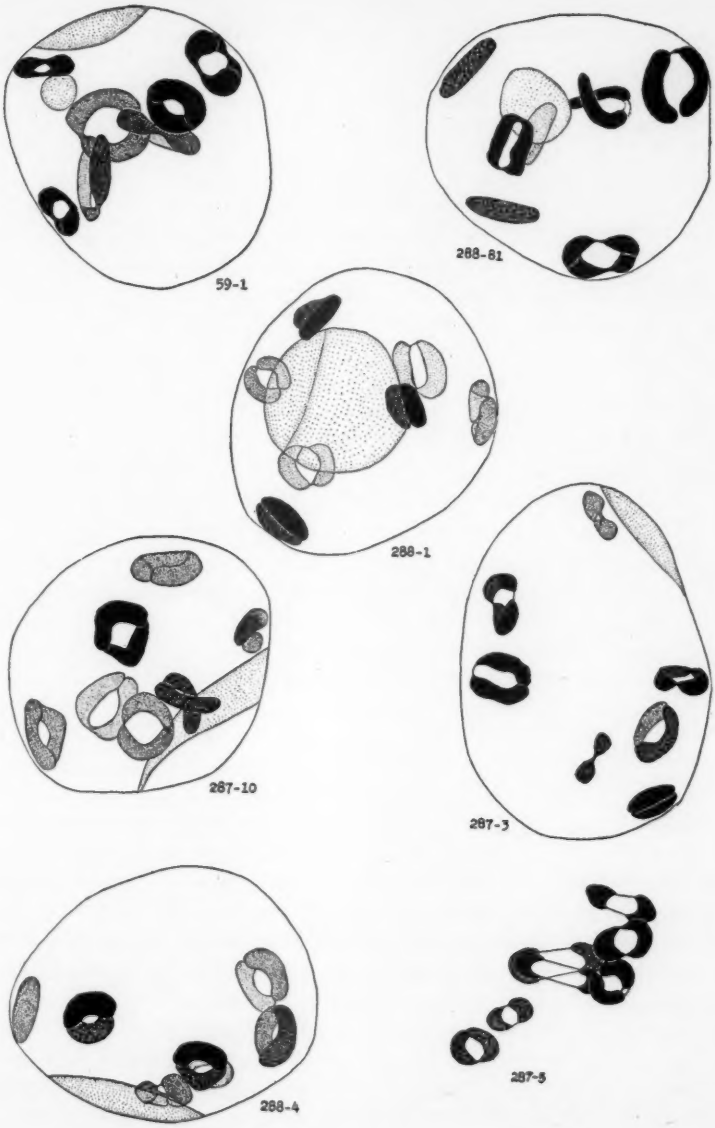

59-1
288-81
288-1
287-10
287-3
288-4
287-5

TABLE 14

Phenotypic frequencies in cultures from self-pollinated plants with splashed bud-cones and of backcrosses to red.

CULTURE	SPLASHED	RED	PARENT
A. self-pollinated			
296	83	27	180– 1
574	36	54	291– 1
575	36	28	–31
576	38	28	292– 9
577	6	6	295–21
581	9	26	296– 7
582	8	4	–10
583	15	28	–11
584	2	36	–12
585	3	15	–15
587	10	11	–16
589	8	15	–17
591	8	11	–20
594	7	16	–33
Total	269	305	
B. splashed×red			
580	17	47	296– 7
586	18	25	–15
588	18	19	–16
590	18	21	–17
593	26	19	–21
595	1	4	–33
Total	98	135	

INTERPRETATION OF DATA

Yellow flower color has long been known to be dominant to sulfur, and the factor pair *S–s* is generally used by Oenothera geneticists to represent the relationship of these colors. In the crosses between *Oe. franciscana* and "*franciscana sulfurea*," there is always a deficiency in the number of functioning gametes which carry the factor *s*. The gametic ratios obtained in F_1 plants with pairing chromosomes were 2133: 821(2.6:1) for the eggs and 370:180 (2.1:1) for the pollen (tables 3, 4, 5, and 6). Gametophyte competition such as described by RENNER (1921) might explain the deficiency of the factor *s* in functioning eggs, and differential pollen-tube growth might be responsible for the deficiency of *s* in functioning sperms. Neither point was checked by direct experiment.

The dwarf stature of sulfur-dwarf is recessive to the tall stature of

franciscana and "*franciscana sulfurea.*" This relationship between the two statures will hereafter be represented by the factor pair *D-d*. Gametes carrying *D* and *d* respectively are produced in approximately equal numbers in F_1 plants with pairing chromosomes. The observed frequencies were 575:505 (1.1:1) for the eggs, and 144:139 (1.03:1) for the sperms (tables 3 and 4).

The green bud-cones of "*franciscana sulfurea*" are dominant to red. The relationship between these bud colors will be represented by the factor pair G_r-g_r[5]. The factor G_r is always associated with a pollen lethal. The gametic ratios of this factor pair will be discussed in another connection.

Splashed bud-cones are dominant to red, but the character is not discernible in the presence of G_r. This relationship is shown by the presence of plants with splashed bud-cones in progenies of green-budded plants that were heterozygous for red bud-cones (tables 9, 10, 11, and 12), and by the presence of red-budded plants in progenies of plants with splashed bud-cones (table 14). No plants were found that bred true for splashed, indicating a possible association with a lethal factor. The relationship between splashed and red bud-cones will be represented by the factor pair S_{pl}-s_{pl}.

The chromosome complexes

Certain F_1 plants from crosses between *Oe. franciscana* and "*franciscana sulfurea*" have seven pairs of chromosomes. This complete pairing of chromosomes is believed to indicate that *franciscana* has seven chromosomes (functioning together as a complex) which are the homologs of seven chromosomes constituting one of the complexes of "*franciscana sulfurea.*" The complexes with pairing chromosomes will be called *franciscans* in *franciscana* and *s-d-franciscans* in "*franciscana sulfurea*" as the latter carries factors for sulfur-colored flowers and dwarf stature. All functioning pollen and some of the functioning eggs of "*franciscana sulfurea*" are *s-d-franciscans* as shown by reciprocal crosses with *franciscana* and with sulfur-dwarf. Sulfur-dwarf is homozygous for this complex.

The other complex of "*franciscana sulfurea*" will be called *sulfurens*. It produces a circle of ten chromosomes and two pairs with *franciscans* and with *s-d-franciscans*, with which it must therefore have two chromosomes (and only two) in common (that is, homologous).

Oenothera franciscana may produce a second complex which forms a circle of four chromosomes with *s-d-franciscans* as found in F_1 plant 58–1 and some of its descendants.

[5] The author follows RENNER (1925) in reserving the factor pair *R-r* [used by SHULL (1923a) for bud color] to denote nerve (vein) color, for which it had previously been used by HERIBERT-NILSSON. RENNER's factor S_r denotes a dominant red bud-color.

Linkage relationships

Oenothera "franciscana sulfurea" produces only two types of gametes, *sulfurens* (carrying factors for sulfur-colored flowers, tall stature, green bud-cones, and a pollen lethal) and *s-d-franciscans* (carrying factors for sulfur-colored flowers, dwarf stature, and red bud-cones). No recombinations of the factors for stature, bud color (red versus green) and the pollen lethal have been found in the gametes of *"franciscana sulfurea"* as shown by inbred and out-cross cultures.

First generation plants with green bud-cones in the cross *"franciscana sulfurea"* × *franciscana* produce for the most part the two parental types of gametes. These gametes are *sulfurens* (carrying factors for sulfur-colored flowers, tall stature, green bud-cones, and a pollen lethal) and *franciscans* (carrying factors for yellow flowers, tall stature, and red bud-cones). No recombinations between bud color and the pollen lethal were obtained. Between bud color and flower color, however, some recombinations occurred. The observed gametic ratios were $27G_r-S:274G_r-s:979\ g_r-S:46\ g_r-s$ for the eggs, and $308\ g_r-S:4g_r-s$ for the sperms (tables 10 and 11). Of the eggs carrying the pollen lethal and factor for green bud-cones, 9 percent represent recombinations with the factor for yellow flowers, while 4.5 percent of the eggs carrying the factor for red bud-cones were recombinations with the factor for flower color, and but 1.28 percent of the sperms represent recombinations between these factors.

In *sulfurens* of *"franciscana sulfurea"* there is also a factor for splashed bud-cones (S_{pl}) closely associated with G_r and s. In F_1 plants with green bud-cones (*sulfurens·franciscans*), 3.5 percent of the eggs carrying g_r represent recombinations with S_{pl} (gametic ratio is $989\ g_r-s_{pl}:36\ g_r-S_{pl}$, table 10) and 3.5 percent or less[6] of the sperms carrying g_r represent recombinations with S_{pl}. In the same egg cells (carrying g_r) there was 7 percent recombination between S_{pl} and S (gametic ratio $953\ S_{pl}-s+s_{pl}-S:\ 72\ S_{pl}-S+s_{pl}-s$).

The percentages of recombinations between these three factors might appear to be a function of a linear order of the three genes in a single chromosome. In this case the order of the genes would be:

$$\underset{\text{(region 1)}}{s_{pl}\quad(3.5)\quad g_r} \qquad \underset{\text{(region 2)}}{(4.5)\quad s}$$

[6] The plants in some cultures listed in table 11 were not in their best condition at the time notes were taken, and some plants listed as having splashed bud-cones may have been red but with the pigmentation poorly developed as is often the case when plants first come into flower. Plants for which the classification was difficult are listed in the tables under "splashed" and followed by a question mark (?).

The observed crossover frequencies would then be: 948 non crossovers ($s_{pl}-g_r-S$), 31 single crossovers in region 1 ($S_{pl}-g_r-S$), 41 single crossovers in region 2 ($s_{pl}-g_r-s$), and 5 double crossovers ($S_{pl}-g_r-s$) (table 10). Expressed in percentages, the frequencies of crossing over are 3.02 singles in region 1, 4.00 in region 2, and 0.49 doubles. There can thus be no interference between crossing over in regions 1 and 2.

No crosses were made in which the associations of factors for flower color ($S-s$) and for stature ($D-d$) could be studied in plants of the constitution *sulfurens·d-franciscans*. But since $s_{pl}-g_r-d$ are almost completely associated in *sulfurens·s-d-franciscans*, and since $s_{pl}-g_r-S$ are very closely associated in *sulfurens·franciscans*, it may fairly be concluded that *s* and *d* are actually closely associated in these hybrids.

In *franciscans·s-d-franciscans*, however, *s* and *d* are completely independent. In the eggs there was 47.4 percent recombination between flower color and stature (gametic ratio 568 $D-S+d-s$:512 $D-s+d-S$, table 3), while in the sperms there was 51.6 percent recombination (gametic ratio 127:156, table 4). The independence of *s* and *d* is further borne out by the segregation of F_3 cultures (tables 7 and 8).

In considering the differences in association of *s* and *d* in these crosses, it must be borne in mind that *franciscans·s-d-franciscans* has all pairing chromosomes, while *sulfurens·franciscans* and *sulfurens·s-d-franciscans* each have ten chromosomes in a circle. It is reasonable to suppose that the linkage of factors in the latter hybrids is due to the association of chromosomes in a circle.

Gametophyte competition

RENNER (1921) has shown that in many cases in which two dissimilar complexes are segregating in megasporogenesis, the megaspores receiving one complex have a distinct advantage over the other type of megaspore in their ability to develop into gametophytes (embryo-sacs). This superiority of the first complex may not hold when it is competing against a third complex.

Competition of this nature is to be seen in crosses involving *Oe.* "*franciscana* and *franciscana sulfurea*." In the "*franciscana sulfurea*" plant used in the crosses of 1923, the frequencies of the two types of complexes functioning as eggs were 45 *sulfurens* and 15 *s-d-franciscans* (culture 59 in table 1). In the F_1 plant 59–3, involving the same *sulfurens* complex against *franciscans*, the gametic ratio was 21 *sulfurens*:25 *franciscans* (culture 180 in table 9). In F_2 plants of culture 180, the gametic ratio was 104 *sulfurens*: 114 *franciscans*. These data are interpreted as indicating that the *sulfurens*

used in 1923 had a marked superiority over *s-d-franciscans* and none over *franciscans*.

In the cross of 1925, the gametic ratio in the "*franciscana sulfurea*" plant used was 203 *sulfurens*:256 *s-d-franciscans* (culture 288 in table 1). In F_1 plants involving the same *sulfurens* against *franciscans*, the gametic ratio was 122 *sulfurens*:232 *franciscans*. In this case both *franciscans* and *s-d-franciscans* are superior to *sulfurens*, but *franciscans* is stronger than *s-d-franciscans* as in the previous case.

The *sulfurens* in the cross of 1923 may have been the original type which formed a circle of twelve with *s-d-franciscans*, while that used in 1925 had become altered to form a circle of ten. Such a difference might account for the divergent results in the crosses of 1923 and 1925.

APPLICATION OF THE BELLING HYPOTHESIS

HÅKANSSON (1928) and DARLINGTON (1929) have shown that the hypothesis (BELLING 1927) of attraction of chromosomes into circles by rearrangements (translocations) of homologous ends will give a mechanical explanation of most of the genetical and cytological phenomena of the Oenotheras. It is not the purpose of the present paper to review the application of BELLING's hypothesis to the Oenotheras, but the hypothesis offers an alternative explanation of the linkage relationships reported above which is in many respects superior to that afforded by the presence of the genes in a single chromosome.

In order to avoid the use of permanent names for the ends of the chromosomes until more information is available concerning the homologies in different species, the homologous portions of the chromosomes in the accompanying diagrams (plate 4) are indicated by differences in shading.

Figure 1 in the diagrams represents the chromosomes of an F_1 plant with green bud-cones (*sulfurens·franciscans*) of the cross "*franciscana sulfurea*" × *franciscana*. It will be noted that each chromosome part is present in the diploid condition. In pairing chromosomes, the parts have identical arrangements; but of the chromosomes in the circle, no two have the same combination of parts. The right end of any chromosome in the ring has its counterpart in the left end of the chromosome on its right, while the counterpart of the left end of the first chromosome is the right end of the chromosome to its left. It is this arrangement of homologous portions of the chromosomes, according to the BELLING hypothesis, which brings the chromosomes into the circle by the attraction between homologous parts.

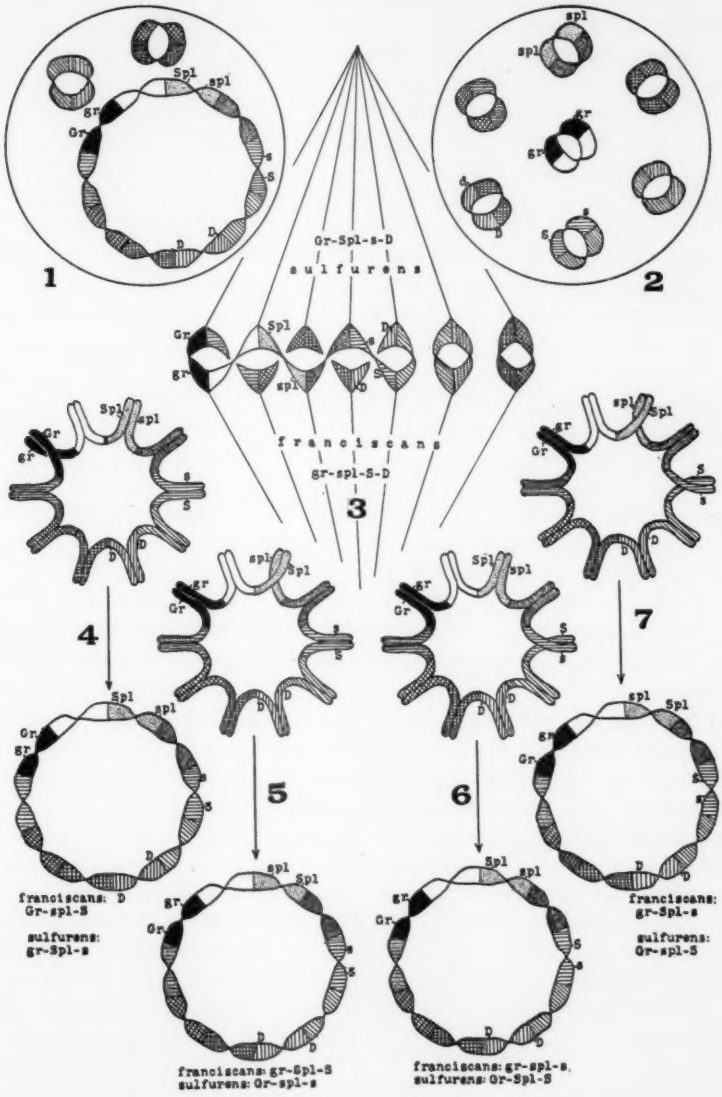

1
2
Gr-Spl-s-D
sulfurens
franciscans
gr-spl-S-D
3
4
5
6
7
franciscans: D
Gr-spl-S
sulfurens:
gr-Spl-s
franciscans: gr-Spl-S
sulfurens: Gr-spl-s
franciscans: gr-spl-s,
sulfurens: Gr-Spl-S
franciscans:
gr-Spl-s
sulfurens:
Gr-spl-S

Figure 2 represents the chromosomes of an F_1 plant with red bud-cones (*franciscans·s-d-franciscans*) in which all the chromosomes pair.

Figure 3 illustrates the method of separation of the chromosomes of figure 1 in heterotypic metaphase. CLELAND discovered that the chromosomes in a circle in Oenothera regularly separate by a zig-zag arrangement of the chromosomes on the metaphase plate, so that alternate chromosomes pass to the same pole. BELLING's hypothesis gives a mechanical explanation of the presence of the chromosomes of one complex in the alternate positions in the circles which necessitates the zig-zag arrangement of chromosomes on the spindle to insure the separation of homologous chromosome parts. It has long been known from genetic data that the paternal and maternal gametes separate as a whole in the reduction division of some Oenotheras.

The positions of the genes in the chromosomes have been chosen arbitrarily. The scheme requires that the genes be in different chromosome parts, but there is no method of determining the position of any gene in the circle with the present data.

It will be noted that the genes will appear to be completely linked even though they lie in different chromosomes, unless there are interchanges (crossovers) between homologous chromosome parts. Such crossovers are represented schematically in figures 4, 5, 6, and 7.

Crossovers of this type do not alter the chromosome configurations, but the allelomorphs present in the two complexes are exchanged. Figure 4 represents a crossover involving $G_r - g_r$ in which G_r passes into *franciscans* and becomes associated with s_{pl} and S. The same association of $G_r - s_{pl} - S$ can be obtained by crossovers involving s_{pl} and s simultaneously (figure 7), but in the latter case $G_r - s_{pl} - S$ would be in *sulfurens*.

In this scheme, percent of crossing over would not be a function of the distance between the two genes appearing as recombinations, but a function of the distances between each of the genes and the respective breaks in the chromosomes (that is, the points at which the chromosomes cease to be homologous to the members on their left and become homologous to the chromosomes on their right). Recombinations between G_r and S_{pl} should be equal to the frequency (a) of crossovers between G_r and the break in that chromosome, plus the frequency (b) of crossovers between S_{pl} and the break in that chromosome, less the frequency (ab) of simultaneous crossovers between these factors and their corresponding "breaks." Similarly, if c is the frequency of crossovers between s and the corresponding break, the number of recombinations between s and S_{pl} must be $b+c-bc$, and between s and G_r must be $a+c-ac$. Crossovers in chromosome

parts lying between any two genes have no effect on the recombinations of these genes. By substituting the observed frequencies of recombinations in each of these equations and solving for a, b, and c, one finds the following values for crossing over between the genes and the corresponding breaks: $G_r = 0.5$ percent, $S_{pl} = 3.0$ percent, and $s = 4.0$ percent.

Interference in crossing over would have little effect on the frequencies of any crossover class, if crossing over occurs in the manner just described. "Double" crossovers are comparatively rare, and the same combination of genes is obtained by a different single crossover.

In plants of the constitution *sulfurens·s-d-franciscans*, G_r, S_{pl}, and *D* have been found to be closely linked with no recombinations occurring between G_r and *D*, while in *sulfurens·franciscans* G_r, S_{pl} and *s* are closely linked, but in *franciscans·s-d-franciscans*, *d* and *s* are independent. These results are easily explained by the scheme just described if one assumes that G_r and *D* both lie comparatively close to the breaks in their respective chromosomes. These positions would prevent recombinations in *sulfurens·s-d-franciscans* in which the chromosomes are in a circle, and would permit free recombination in *franciscans·s-d-franciscans* in which the chromosomes pair.

Similar cases in Oenothera

Crossing over between homologous chromosomes, as described above, may be the cause of the appearance of such mutations as *biennis sulfurea* from *Oe. biennis*, *nanella* from *Oe. Lamarckiana*, and *formosa* from *Oe. pratincola* (strain E).

In the first case, *Oe. biennis* is known to be balanced for sulfur[7] (OEHLKERS 1924), and the mutation, *biennis sulfurea*, still has the peculiar chromosome configuration of the parent form (CLELAND 1926).

The second case fulfills the same two conditions. *Lamarckiana* is balanced for *nanella* (carried in *gaudens*). I do not know if the chromosome configuration of *nanella* has been observed, but there is excellent genetic evidence that *nanella* forms *velans* and *gaudens* complexes which are identical to those of *Lamarckiana* except for the presence of the *nanella* factor in *velans* (RENNER 1925).

Oenothera pratincola (strain E) is known to be balanced for revolute leaves (COBB and BARTLETT 1919, COBB 1921). Mutation *formosa* (revolute-leaved) and *pratincola* have the same chromosome configuration

[7] The *albicans* complex of *biennis* carries sulfur, but due to the presence of balanced zygotic lethals, *albicans* does not appear in homozygous condition, and the dominant yellow allelomorph in *rubens* masks the presence of sulfur.

(KULKARNI 1928). Thus again, the two conditions required by the method of crossing over described above are fulfilled. In this last case, however, the genetic data of Doctor BLANCHARD (COBB 1921) do not entirely agree with present predictions. Plants with the complexes of *formosa* (one, however, recovered from a cross with *pratincola*-C) but heterozygous for revolute behave in their breeding as if the factor for revolute were in a chromosome pair separate from the large complexes. No free pair is expected if the above interpretation is correct.

Similar cases in Drosophila

As a result of X-ray treatment, DOBZHANSKY and STURTEVANT (1930) have obtained reciprocal translocations between the second and third chromosomes of *Drosophila melanogaster*. While the cytology of the reduction divisions has not been completed, the somatic pairing of chromosomes in the Diptera gives a good indication of the configuration that the chromosomes must assume during reduction. In somatic cells of flies having one normal second, one normal third, and the two reciprocally translocated chromosomes, the four chromosomes often form a ring. Genetic evidence shows that alternate chromosomes of the ring pass to the same pole in the majority of cases, thus agreeing with the cytological findings for Oenothera.

As the paper of DOBZHANSKY and STURTEVANT includes a discussion of the relation of their findings to the problems of Oenothera genetics, it is necessary here only to point out that there is increasing genetical and cytological data in support of BELLING'S hypothesis for ring formation. DOBZHANSKY and STURTEVANT did not collect linkage data that is completely comparable to that reported above for Oenothera, but it is evident that such data would be obtained if the linkage of genes in three chromosome-halves were studied together.[8]

ACKNOWLEDGMENT

The studies on Oenothera reported above were undertaken under the direction of Professor H. H. BARTLETT and Professor B. M. DAVIS. I am

[8] Since this paper went to press I have studied the crossing over between the four limbs of DOBZHANSKY'S reciprocal translocations *A*, *B*, and *C*. All gave the predicted results and agree with the linkage observed for $S_{pl}-b_r-s$ reported above for Oenothera. For example: in translocation II–III B, B_l lies to the right of the "break" in chromosome II, p^p to the left of the "break" in III and s_t to the right. From the linkage data, the three genes might lie in a single chromosome in the order $s_t-B_l-p^p$ with 6.39 percent single crossover in region 1, 1.86 percent in region 2 and 0.40 percent double (total 2051 flies) when only 0.15 percent double would be expected if there were no interference. In the Oenothera cross reported above there were 0.49 percent double when only 0.17 percent would be expected with no interference.

indebted to Professor DAVIS for the use of his cultures and especially for seed of crosses made by him. I am indebted to Mr. C. O. ERLANSON, Mr. W. C. STEERE, and Miss ELIZABETH GRIFFITHS for assistance in the cytological part of the investigation.

SUMMARY AND CONCLUSIONS

Oenothera franciscana may produce two chromosome complexes which together form a ring of four chromosomes. One of these complexes is called *franciscans*.

Hybrid "*franciscana sulfurea*" produces a complex of which all the chromosomes are homologous to those of *franciscans*, since plants composed of these two complexes give seven pairs of chromosomes. This complex of "*franciscana sulfurea*" differs from that of *franciscana* by recessive factors for dwarf stature and sulfur-colored flowers and is called *s-d-franciscans*.

The second complex of "*franciscana sulfurea*" is called *sulfurens*. *Sulfurens* forms a circle of ten chromosomes and two pairs with each of the two *franciscans*-types of complexes.

In crosses involving the three complexes just named, four factor pairs were concerned. These factors were $D-d$, $S-s$, G_r-g_r (a dominant green bud color), and $S_{pl}-s_{pl}$ (splashed bud color, dominant to red).

In *sulfurens·s-d-franciscans* with a circle of ten chromosomes, G_r S_{pl}, and D are closely linked. In *sulfurens·franciscans* with a circle of ten chromosomes, G_r, S_{pl}, and s are closely linked. But in *franciscans·s-d-franciscans* which has all pairing chromosomes, s and d are completely independent. This relationship is believed to show that the linkage of D and s to G_r and S_{pl} in the first two hybrids is due to the association of a large number of chromosomes in a circle in each.

The interpretation that the linkage of G_r, S_{pl}, and s in *sulfurens·franciscans* is due to the presence of the three genes in one chromosome is inadequate in that it does not account for the excessive number of observed "double crossovers."

Crossing over between homologous parts of dissimilar chromosomes in the circle of ten in *sulfurens·franciscans*, however, does account for all the observed genetic behavior. Crossing over of this type presupposes the correctness of BELLING'S hypothesis for the attraction of chromosomes into rings due to homologous chromosome-ends.

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A SPLIT IN CHROMOSOMES ABOUT TO ENTER THE SPERMATID (*PARATETTIX TEXANUS*)[1]

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MULLER's discovery of fractional mutations which affect but one side or portion of the body in Drosophila developing from eggs fertilized by X-rayed sperm has been interpreted as indicating that chromosomes within the spermatozoon must have been split (MULLER 1927). In view of this it seems worth while to place on record an observation on anaphase chromosomes coming out of the secondary spermatocyte division in a male of *Paratettix texanus*.

This individual was produced parthenogenetically (See M/M ♂ in Item 76, NABOURS and FOSTER 1929), but aside from the fact that its chromosomes in the spermatogonia have a tendency in many cases to lie with homologues together (figures 1 and 2) and also possibly that the halves of the sex diad may cling very loosely together in the anaphase of the first maturation (figures 3 and 4), there is nothing about it which in any way differs from the ordinary bisexually produced male. That it was normal is to be inferred from the fact that it became the sire of some 298 young (data from Doctor NABOURS). Since this individual was of parthenogenetic origin its chromosomes have been studied rather intensively (ROBERTSON 1930).

In the spermatogonia (figures 1 and 2) there are six pairs of autosomes and a single X-chromosome. The primary spermatocytes (figures 3 and 4) have the usual 6 tetrads and the X-chromosome. The tetrads exhibit their four parts if properly oriented (figure 3), likewise, the sex chromosome its two parts as it travels toward one of the poles.

The secondary spermatocyte metaphase has either 6 or 7 diads (figures 5 and 7). In the beginning and later anaphases (figures 8, 6, 9) some of the secondary divisions show most of the daughter chromosomes split (figures 8 and 9). Of course part of these may not be oriented in a way to exhibit the split (Nos. 2x and 6 in figure 8). All the daughter chromosomes in figure 10, a late anaphase about to enter the telophase, show a perpendicular split.

[1] Contribution No. 123, Department of Zoology, KANSAS AGRICULTURAL EXPERIMENT STATION, Manhattan, Kansas.

All the drawings were made to the same scale. In size the daughter chromosomes showing the split (figures 8–10) are about one-half that of those in secondary spermatocyte metaphases and one-fourth that of tetrads (figures 3 and 4). The same is true of the cells containing them. From this and from the sequence of stages as shown by the cysts in the follicle it may be concluded that these chromosomes are groups about to enter the spermatid. If so, it seems to follow that in Paratettix and Tettigidae in general (?) most likely, the chromosomes entering a spermatozoon must be split.

The time of origin of this split and the time when the chromonemata must have become separated (disentangled) from each other should be considered in order to account for the conditions found in figures 8 to 10. In the Tettigidae it has been shown (ROBERTSON 1931) that homologous chromosomes are already split when they enter upon parasynapsis. It is thought that this split in each homologue must have originated in the resting period prior to the last spermatogonial division. In the prophases of that division the daughter chromonemata, in each half chromosome, may have succeeded in getting partially separated, but from the appearance of the split chromonema in the telophase of this division the convolutions and crimps in its daughter chromonemata show that they must have lain very closely together when the condensation and crimping of the prophase overtook them. Evidence is shown (ROBERTSON 1931) that the telophase of this division, leading to synapsis, effects the completion of the separation of the daughter chromonemata.

Parasynapsis then is thought to begin by the intimate approximation of the daughter threads of each homologue and to be continued and completed by the approximation of the homologues themselves at the usual "bouquet" and "zygotene" period. This results in the diplotene thread which may therefore be considered to consist of four completely distinct threads, but which exhibits a double-threaded condition due to this, that the parasynapsis between the daughter threads in each homologue on account of the longer period for pairing may be more intimate than the pairing between the homologues.

The thread, therefore, that enters the "diffuse" ("resting") period may be considered made up of four completely separated threads. When the tetrad is formed and emerges from this stage, in the prophase that follows each of these strands may crimp independently and with the condensation and matrix (?) formation that accompanies it result in the tetrad of the first spermatocyte.

But in the "diffuse" or "resting" period opportunity it would seem might be afforded for each of the four strands to split. The daughter chromone-

mata may, however, in the crimping and condensation process that follows have to crimp together. Their separation might be effected in the interphase (telophase and semi-resting period) that intervenes between the first and second spermatocyte division. In the prophase of the second division the daughter chromonemata in each chromatid of a "diad" may be able to crimp separately as the chromatid approaches the metaphase. In this way we might account for the split in the daughter chromosomes (chromatids) of the second spermatocyte anaphases as shown in figures 8 to 10.

The writer is indebted to Doctor R. K. NABOURS for this material, and to both Doctor NABOURS and Dean L. E. CALL for financial support while working it up.

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ON THE ORIGIN OF PARTHENO-PRODUCED MALES IN TETTIGIDAE (APOTETTIX AND PARATETTIX)[1]

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Parthenogenesis in this group results in a preponderance of females, males are rare. Among 5326 parthenogenetically produced offspring in *Apotettix eurycephalus* only 13 have been males (NABOURS 1925) and among 582 in *Paratettix texanus* 2 have been males (NABOURS and FOSTER 1929). A study of the chromosomes of partheno-produced compared with biparentally produced individuals (ROBERTSON 1930) and the genetic work of NABOURS (1919, 1925, 1929) and NABOURS and FOSTER (1929) have led to the conclusion that parthenogenesis in the Tettigidae is a case of development following the throwing off of but one polar body and therefore of the diploid type. If this be true the occasional production of males and the presence as will be shown later of male-producing lines of females may be accounted for in one of three ways two of which seem the more probable:

1. Through non-disjunction among the four parts (chromatids) of the sex tetrad at the first polar division. The tettigid type of tetrad would be quite favorable for this if it may be supposed that the behavior of tetrads in the female is similar to that in the male. In other grasshoppers they behave alike (MCNABB 1928, figures 41–1, 2, 3). Each tetrad arises from a pair of homologous chromosomes that were split before entering synapsis (ROBERTSON 1916, 1917, 1919, 1931a). On coming out of synapsis, in the case of each pair, the (split) members, or what result presumably after crossing over is effected, disjoin from each other, beginning at the proximal ends, the distal ends remaining attached, until they are 180 degrees apart, thus forming a four-part structure (ROBERTSON 1931a). (In the case of the cross-type tetrad of which there are a few, disjunction may still be in progress at the region of the transverse arms.) In this condition the tetrad comes into the first maturation metaphase, with one split—or rather two-part—member pointed toward one pole and the other member pointed toward the opposite pole (ROBERTSON 1916, figures 69–71, 75–77, 1917, figures 14–21, 1930, figures 60–63, and 1931b, figure 3). In the anaphase of the first division the halves of a diad gap apart (ROBERTSON 1916, figures

[1] Contribution No. 124, Department of Zoology, KANSAS AGRICULTURAL EXPERIMENT STATION, Manhattan, Kansas.

122, 123, 132–135). The sex diad of the partheno-produced male (1930, figures 63, 1931b, figures 3 and 4) and his male offspring (1930, figure 80) (though the sex-chromosome here comes from the mother) has its parts very loosely attached as it travels toward one of the poles. It might be suspected that at the metaphase of the first polar body formation the same loose connections among the four parts in at least some of the Apotettix and Paratettix parthenogenetic lines might result in the occasional 3 to 1 distribution of the 4 sex chromatids, thus leaving one in the egg with the six autosome diads.

2. and 3. The second and third possibilities are based upon the fact that the sex-chromosome (in the male at least) seems to be precocious in its tendency to divide although it may lag during the completion of the anaphase. If therefore one of the maturation divisions be abortive, usually, for all of the tetrads, resulting in the retention of complete diploidy, it might occasionally happen that, due to the precociousness of the sex tetrad in division, one-half of the sex-chromatin might be included in the polocyte. Such behavior at either division would result in a single X-chromatid with diploid autosomes. It seems more in keeping with what we know about maturation in the Tettigidae from both genetic and cytological standpoints that the second division, rather than the first, might be abortive. If the first were so, the autosome tetrads might not divide, only the sex tetrad, and the second division would have to be equational for the sex diad but reductional for the autosome tetrads. If, instead, as we would rather think, the first division be reductional for all of the tetrads the second would have to be abortive for the autosome diads but occasionally equational for the sex diad.

Either the last mentioned method or the non-disjunctional method would agree with the breeding results of NABOURS (1925, 1929, etc.) which suggest that the mechanism for parthenogenesis, whatever it be, should be one that might provide simultaneously for either parthenogenesis or bisexual reproduction.

There are a few other possibilities (sometimes suggested) that should be mentioned in connection with male production in the Tettigidae.

Non-disjunction in one of the X-chromosomes it is thought might have occurred in the oogonial divisions prior to oocyte formation. But if such did occur, would a cell with 13 chromosomes develop into an egg? Or, supposing it should, the first maturation would result in either 7 diads or 6 diads remaining in the egg. In the latter case probably no viable embryo would result; in the former the embryo would be a female. Pre-maturation non-disjunction could result only in females.

BRIDGES (1916) found in Drosophila that an extra Y-chromosome was a factor in bringing about the non-disjunction of the two X's at maturation. It might be thought that some supernumerary deficient X-chromosome similar to what ROBERTSON (1917) found in *Tettigidea parvipennis* might play the part of the Y-chromosome in connection with the distribution of the four parts of the sex tetrad. But if so it would have to travel inward with the single X-chromatid,thus remaining in the egg, and of course would be expected to be present as two extra, deficient chromosomes in the cells of such males. But no such supernumerary bodies have so far been found.

It might also be assumed that there are triple-X females in these male-producing strains. At the first maturation, reduction in the sexad might result in two, three, or four sex-chromatids remaining in the egg. If the quadruple-X eggs were non-viable the XX and XXX classes would be left. The last class is not viable in Drosophila (BRIDGES 1916). For male production a second division abortive for the autosome diads but occasionally effective for the sex diad or triplad would have to be assumed. Such triple-X females would supply the demand for a male-producing strain.

That there is evidence for some of the possibilities that have been suggested above being handed on in certain lines may be seen by tracing the ancestry of the thirteen partheno-produced males shown in the project on Apotettix (NABOURS 1925). There apparently is a concentration of the production of these males about NABOURS' "item" 5298. At least seven males from six different items (5007, 5008, 5294, 5295, 5296, 5297) may be traced through from three to five parthenogenetic generations directly to the family of this item, which was produced by a single KK female. This female's line in turn runs through three brother-sister matings, 543, 383, to "item" 100, the male for which was from 502 and the female from 592. In the ancestry of each of the remaining six males (from items 5101, 5131, 5327, 5415, 5418) either item 5298 or the matings (items) immediately back of this occur many times over, and in the majority of their ancestral lines, though it may have in some cases to be traced through many generations, both bisexual and parthenogenetic.

With one exception every item or series of parthenogenetic items which produces a male is preceded by one or more inbred generations. The exception is in item 5415 which was produced by a female from the bisexually produced item, 271. However it may be noticed that item 271 came from a male and a female whose ancestral items, for the most part bisexual, eight generations back contained in the two or three generations preceding each either the item 5298, 543, or 383.

The fact that item 271 could produce a male-producing female, together

with the cases of inbreeding preceding parthenogenetic lines that produced males, would suggest a peculiarity in the X-chromosome itself that evidently came in through the male as well as the female. If so, it would seem that some peculiarities of the sex-chromosomes may have the chief part to play (through non-disjunction possibly) in occasional male-production rather than some cytoplasmic peculiarity such as an abortive division. But if it did it would not necessarily rule out the possibility that a combination of both might occur.

An examination of the sex chromosomes themselves in partheno-produced males (ROBERTSON 1930, figures 21–25, 48–63, 1931b, figures 1–4) reveals so far nothing exceptional in their structure unless it be in the somewhat precocious tendency toward division apparent in diploid cells (1930, figures 25, 48, 49, 52, 56, 59) and in the more loose connection between members of the diad as it travels toward the pole in the first maturation (1930, figure 63, 1931b, figures 3 and 4).

The writer wishes to acknowledge his indebtedness to Doctor R. K. NABOURS for the opportunity to work upon this material and to both Doctor NABOURS and Director L. E. CALL for financial support during the progress of this and the work upon which it is based.

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INBREEDING AND LINKAGE*

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When a heterozygous population is self-fertilized or inbred the ultimate result (apart from effects of mutation) is complete homozygosis. The final proportions of the various genotypes are usually independent of the system of inbreeding adopted, although, as JENNINGS (1916) and others have shown, the speed at which equilibrium is approached is greater in the case of self-fertilization than of brother-sister mating, and so on.

If however the population be heterozygous for linked genes, the final proportions depend on the system of mating, for crossing over can only occur in double heterozygotes, and the proportion of double heterozygotes falls off at a different rate in different mating systems. JENNINGS (1917) stated that he "would find it a relief if someone else would deal thoroughly with the laborious problem of the effects of inbreeding on two pairs of linked factors." This is the object of the present paper. ROBBINS (1918) solved the problem in the case of self-fertilization.

In what follows we employ a direct method to obtain the final proportions of the population. The rate of approach can be calculated, but this is a very laborious process, and always involves the irrational roots of quadratic, sometimes those of quartic or higher equations. In each case we shall suppose that the number of dominant and recessive genes of each type in the population is equal throughout the progress of the inbreeding. This enormously simplifies the mathematics. Thus a system of 55 equa-

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tions described by JENNINGS (1917) is at once reduced to 22. This restriction is later removed.

SELF-FERTILIZATION

This problem has been solved by ROBBINS, but the shorter solution here given serves to illustrate our method. Consider the results of crossing *AABB* and *aabb*, where *A* and *B* are linked. The crossover values on the two sides of a hermaphrodite are taken as 100β percent and 100δ percent, so that the two gametic series given by *AB.ab* are:

$$(1-\beta)AB:\beta Ab:\beta aB:(1-\beta)ab.$$
$$(1-\delta)AB:\delta Ab:\delta aB:(1-\delta)ab.$$

For the sake of symmetry we suppose the original population to be entirely *AB.ab*. Then in the nth generation which is self-fertilized, let the 10 zygotic types occur in the proportions:

C_n *AABB* and *aabb*.
D_n *AAbb* and *aaBB*.
E_n *AABb*, *AaBB*, *Aabb*, and *aaBb*.
F_n *AB.ab*.
G_n *Ab.aB*.

We assume $2C_n+2D_n+4E_n+F_n+G_n=2$, so that $C_1=D_1=E_1=G_1=0$, and $F_1=2$. Clearly $E_\infty=F_\infty=G_\infty=0$, and D_∞ is the final proportion of crossover zygotes. Then considering the results of selfing each generation, we have:

$$\left.\begin{aligned}
C_{n+1} &= C_n + \tfrac{1}{2}E_n + \tfrac{1}{4}(1-\beta-\delta+\beta\delta)F_n + \tfrac{1}{4}\beta\delta G_n \\
D_{n+1} &= D_n + \tfrac{1}{2}E_n + \tfrac{1}{4}\beta\delta F_n + \tfrac{1}{4}(1-\beta-\delta+\beta\delta)G_n \\
E_{n+1} &= \tfrac{1}{2}E_n + \tfrac{1}{4}(\beta+\delta-2\beta\delta)(F_n+G_n) \\
F_{n+1} &= \tfrac{1}{2}(1-\beta-\delta+\beta\delta)F_n + \tfrac{1}{2}\beta\delta G_n \\
G_{n+1} &= \tfrac{1}{2}\beta\delta F_n + \tfrac{1}{2}(1-\beta-\delta+\beta\delta)G_n
\end{aligned}\right\} \qquad (1.1)$$

These equations are derived as follows. The homozygous types when selfed reproduce themselves only, so C_n and D_n contribute only to C_{n+1} and D_{n+1}. *AABb* selfed gives $\frac{1}{4}AABB$, $\frac{1}{4}AAbb$, $\frac{1}{2}AABb$. Hence the contribution of E_n to E_{n+1} is $\frac{1}{2}E_n$. Since there are twice as many classes in the proportion E_n as C_{n+1}, the coefficient of its contribution to C_{n+1} must be doubled, and similarly for its contribution to D_{n+1}. The contributions of F_n and G_n are similarly calculated.

Now put $C_n-D_n=c_n$, $F_n-G_n=d_n$, and the average crossover value,

$\frac{1}{2}(\beta+\delta)=x$. Then subtracting the equations for C_{n+1}, D_{n+1}, and F_{n+1}, G_{n+1}, we have:

$$\left.\begin{aligned} c_{n+1} &= c_n + \tfrac{1}{4}(1-2x)d_n \\ d_{n+1} &= \tfrac{1}{2}(1-2x)d_n \end{aligned}\right\} \tag{1.2}$$

Now choose λ so that $c_{n+1}+\lambda d_{n+1} \equiv c_n+\lambda d_n$ for all values of n.

Then $c_n + \lambda d_n \equiv c_n + \frac{1}{4}(1-2x)d_n + \frac{1}{2}\lambda(1-2x)d_n$

$$\therefore\ \lambda = \frac{1-2x}{2+4x}.$$

Then since $d_\infty = 0$, and $c_1 = 0$, $d_1 = 2$,

$$c_\infty = c_\infty + \lambda d_\infty = c_1 + \lambda d_1 = \frac{1-2x}{1+2x}.$$

Put $y = D_\infty$ (the final proportion of crossover zygotes)

$$\therefore\ C_\infty + D_\infty = 1,\ C_\infty - D_\infty = c_\infty\ \therefore\ y = \tfrac{1}{2}(1-c_\infty).$$

$$\therefore\ y = \frac{2x}{1+2x}. \tag{1.3}$$

Hence the proportion of crossover zygotes is approximately equal to twice the mean gametic crossover value when the latter is small, rising to 50 percent with 50 percent crossing over (see figure 1). The actual proportions of the different zygotic types in each generation can be calculated from equations (1.1). Equations (1.2) are not sufficient. The method of solution is given by ROBBINS, and the principal result in our terminology, putting $p=\beta\delta$, the product of the crossover values, is:

$$\begin{aligned} C_n &= \frac{1-(\frac{1}{2}-x)^n}{1+2x} + \tfrac{1}{2}(\tfrac{1}{2}-x+p)^{n-1} - (\tfrac{1}{2})^{n-1} \\ D_n &= \frac{2x+(\frac{1}{2}-x)^n}{1+2x} + \tfrac{1}{2}(\tfrac{1}{2}-x+p)^{n-1} - (\tfrac{1}{2})^{n-1}. \end{aligned} \tag{1.4}$$

Thus the final proportion of crossover zygotes, D_∞, depends on x only; the rate of approach to this value depends on p. Indeed if crossing over were restricted to one side of a hermaphrodite, as it is to one sex in the higher insects, we should have $p=0$, and no crossover zygotes would appear before F_3. D_n is 0 in F_1, $\frac{1}{2}p$ in F_2, rising sharply to $\frac{1}{2}(x+p-2px+p^2)$ in F_3, and over half way to its final value in F_4. Except in F_2 the figures

depend almost entirely on the mean crossover value. Thus with a mean value of 10 percent we have in successive generations the percentages of crossover homozygotes given in table 1.

TABLE 1

CROSSOVER VALUES PERCENT	F_2	F_3	F_4	F_5	F_6	F_∞
10, 10	0.50	5.405	9.746	12.683	14.462	$16.\dot{6}$
20, 0	0	5.0	9.5	12.55	14.395	$16.\dot{6}$

Hence in a plant propagated by self-fertilization, where new combinations are required after a cross, there is a very great advantage in growing on a large progeny as far as F_3, and rather little advantage in growing it beyond F_4. As will appear later, this is also true when double crossing over is taken into account.

BROTHER-SISTER MATING. SEX-LINKED GENES

Two sex-linked genes provide four types of zygotes in the heterogametic sex, and ten in the homogametic. There are thus forty different types of mating. If we consider the results of an original mating $AABB \times ab$, or $aabb \times AB$, the numbers of A and a genes are unequal, and there is a lack of symmetry in the equations, just as there would be in the case last considered if we did not begin with equal numbers of the allelomorphs. We therefore suppose that at the beginning both reciprocal crosses were made in equal numbers. The crossover percentage is taken as 100β, and $\alpha = 1 - \beta$. The fourteen variables of equations (2.1) refer to the proportions of matings of each type. Under the circumstances considered all matings fall into one of fourteen classes having the same frequency in each generation, which is calculable when we know the frequencies in the preceding generation.

MATINGS	PROPORTION
$AABB \times AB$ $aabb \times ab$	$C_{n+1} = C_n + \frac{1}{2}I_n + \frac{\alpha^2}{4}M_n + \frac{\beta^2}{4}Q_n$
$AAbb \times Ab$ $aaBB \times aB$	$D_{n+1} = D_n + \frac{1}{2}J_n + \frac{\alpha^2}{4}P_n + \frac{\beta^2}{4}R_n$
$AABB \times Ab$ $AABB \times aB$ $aabb \times aB$ $aabb \times Ab$	$E_{n+1} = \frac{1}{4}I_n + \frac{\alpha\beta}{4}(M_n + Q_n)$

$$\left.\begin{matrix} AAbb \times AB \\ AAbb \times ab \\ aaBB \times AB \\ aaBB \times ab \end{matrix}\right\} \quad F_{n+1} = \tfrac{1}{4}J_n + \frac{\alpha\beta}{4}(P_n + R_n)$$

$$\left.\begin{matrix} AABB \times ab \\ aabb \times AB \end{matrix}\right\} \quad G_{n+1} = \frac{\alpha^2}{4}M_n + \frac{\beta^2}{4}Q_n$$

$$\left.\begin{matrix} AAbb \times aB \\ aaBB \times Ab \end{matrix}\right\} \quad H_{n+1} = \frac{\alpha^2}{4}P_n + \frac{\beta^2}{4}R_n$$

$$\left.\begin{matrix} AaBB \times AB \\ AABb \times AB \\ Aabb \times ab \\ aaBb \times ab \end{matrix}\right\} \quad I_{n+1} = E_n + \tfrac{1}{4}(I_n + J_n + K_n) + \frac{\alpha\beta}{4}(M_n + Q_n) + \frac{\beta^2}{4}P_n + \frac{\alpha^2}{4}R_n$$

$$\left.\begin{matrix} AaBB \times aB \\ AABb \times Ab \\ Aabb \times Ab \\ aaBb \times aB \end{matrix}\right\} \quad J_{n+1} = F_n + \tfrac{1}{4}(I_n + J_n + L_n) + \frac{\alpha\beta}{4}(P_n + R_n) + \frac{\beta^2}{4}M_n + \frac{\alpha^2}{4}Q_n$$

$$\left.\begin{matrix} AaBB \times Ab \\ AABb \times aB \\ Aabb \times aB \\ aaBb \times Ab \end{matrix}\right\} \quad K_{n+1} = \tfrac{1}{4}K_n + \frac{\alpha\beta}{4}(P_n + R_n) + \frac{\beta^2}{4}M_n + \frac{\alpha^2}{4}Q_n'$$

$$\left.\begin{matrix} AaBB \times ab \\ AABb \times ab \\ Aabb \times AB \\ aaBb \times AB \end{matrix}\right\} \quad L_{n+1} = \tfrac{1}{4}L_n + \frac{\alpha\beta}{4}(M_n + Q_n) + \frac{\beta^2}{4}P_n + \frac{\alpha^2}{4}R_n$$

$$\left.\begin{matrix} AB \cdot ab \times AB \\ AB \cdot ab \times ab \end{matrix}\right\} \quad M_{n+1} = G_n + \tfrac{1}{2}L_n + \frac{\alpha^2}{2}M_n + \frac{\beta^2}{2}Q_n$$

$$\left.\begin{matrix} Ab \cdot aB \times Ab \\ Ab \cdot aB \times aB \end{matrix}\right\} \quad P_{n+1} = H_n + \tfrac{1}{2}K_n + \frac{\alpha^2}{2}P_n + \frac{\beta^2}{2}R_n$$

$$\left.\begin{matrix} Ab \cdot aB \times AB \\ Ab \cdot aB \times ab \end{matrix}\right\} \quad Q_{n+1} = K_n + \frac{\alpha\beta}{2}(P_n + R_n)$$

$$\left.\begin{matrix} AB \cdot ab \times Ab \\ AB \cdot ab \times aB \end{matrix}\right\} \quad R_{n+1} = L_n + \frac{\alpha\beta}{2}(M_n + Q_n).$$

(These equations are referred to as 2.1.)

As an illustration of how these equations are derived we may take the distribution of K_n in the following generation. The mating $AaBB \times Ab$ gives $AABb$, $Ab.aB$, AB, and aB offspring in equal numbers. Hence in the next generation the matings $AABb \times AB$, $AABb \times aB$, $Ab.aB \times AB$, and $Ab.aB \times aB$ occur in equal numbers among its progeny. Hence K_n contributes to I_{n+1}, K_{n+1}, Q_{n+1}, P_{n+1} as shown. To reduce the equations (2.1) we put:

$c_n = C_n - D_n$, $d_n = E_n - F_n$, $e_n = G_n - H_n$, $f_n = I_n - J_n$, $g_n = K_n - L_n$, $h_n = M_n - P_n$, $i_n = Q_n - R_n$.

$$\left.\begin{aligned}
\therefore\ c_{n+1} &= c_n + \tfrac{1}{2}f_n + \frac{\alpha^2}{4}h_n + \frac{\beta^2}{4}i_n \\
d_{n+1} &= \tfrac{1}{4}f_n + \frac{\alpha\beta}{4}(h_n + i_n) \\
e_{n+1} &= \frac{\alpha^2}{4}h_n + \frac{\beta^2}{4}i_n \\
f_{n+1} &= d_n + \tfrac{1}{4}g_n + \frac{\alpha\beta - \beta^2}{4}h_n - \frac{\alpha^2 - \alpha\beta}{4}i_n \\
g_{n+1} &= \tfrac{1}{4}g_n - \frac{\alpha\beta - \beta^2}{4}h_n + \frac{\alpha^2 - \alpha\beta}{4}i_n \\
h_{n+1} &= e_n - \tfrac{1}{2}g_n + \frac{\alpha^2}{2}h_n + \frac{\beta^2}{2}i_n \\
i_{n+1} &= \tfrac{1}{2}g_n - \frac{\alpha\beta}{2}(h_n + i_n)
\end{aligned}\right\} \qquad (2.2)$$

When $n = 0$, G_n and therefore $e_n = 1$, the other variables are zero. When $n = \infty$, all but C_n, D_n, and consequently c_n vanish. It is required to find the value of c_∞. To do so we have to find values of ϵ, ζ, η, θ, ϕ, ψ, such that

$$c_{n+1} + \epsilon d_{n+1} + \zeta e_{n+1} + \eta f_{n+1} + \theta g_{n+1} + \phi h_{n+1} + \psi i_{n+1} \equiv c_n + \epsilon d_n + \zeta e_n + \eta f_n + \theta g_n + \phi h_n + \psi i_n.$$

Substituting in the above identity the values of c_{n+1}, etc. and equating coefficients of d_n, etc. we have:

$$\left.\begin{aligned}
\epsilon &= \eta \\
\zeta &= \phi \\
\eta &= \tfrac{1}{2} + \tfrac{1}{4}\epsilon \\
\theta &= \tfrac{1}{4}(\eta + \theta - 2\phi + 2\psi) \\
\phi &= \frac{\alpha^2}{4}(1 + \zeta + 2\phi) + \frac{\alpha\beta}{4}(\epsilon - 2\psi) + \frac{2\beta - \beta^2}{4}(\eta - \theta) \\
\psi &= \frac{\beta^2}{4}(1 + \zeta + 2\phi) + \frac{\alpha\beta}{4}(\epsilon - 2\psi) + \frac{\alpha\beta - \alpha^2}{4}(\eta - \theta)
\end{aligned}\right\} \qquad (2.3)$$

Eliminating $\epsilon = \eta = \frac{2}{3}$, and $\phi = \zeta$, we have

$$9\theta = 2 - 6\zeta + 6\psi$$
$$12(\zeta - \psi) = (\alpha - \beta)(5 + 9\zeta - 3\theta).$$
$$12(\zeta + \psi) = 1 + 3(\alpha^2 + \beta^2)(3\zeta + \theta) - 6\alpha\beta\psi.$$

Hence, putting $\beta = x$,

$$\epsilon = \zeta = \tfrac{2}{3}, \zeta = \phi = \frac{3 - 4x}{3(1 + 4x)}, \quad \theta = 2\psi = \frac{2(4x - 1)}{3(1 + 4\beta)}.$$

Hence, for all values of n,

$$c_\infty = c_n + \tfrac{2}{3}(d_n + f_n) + \frac{(3 - 4x)(e_n + h_n)}{3(1 + 4x)} + \frac{(4x - 1)(2g_n + i_n)}{3(1 + 4x)} \quad (2.4)$$

and since $C_n + D_n = 1$, $C_n - D_n = c$

$$\therefore\ y = D_n = \tfrac{1}{2}(1 - c_n).$$

In the case here considered $e_0 = 1$

$$\therefore\ c_\infty = \frac{3 - 4x}{3(1 + 4x)}$$
$$\therefore\ y = \frac{8x}{3(1 + 4x)}. \quad (2.5)$$

This is plotted in figure 1. It will be seen that if two sex-linked genes give 50 percent crossing over in the homogametic sex, the final proportion of crossover zygotes will be 4/9. In order to study the rate at which the final values are approached it is necessary to solve the equations (2.2), and also a corresponding set of seven equations for $C_n + D_n$, etc. This is quite possible. Thus it can easily be shown that

$$(h_{n+2} - i_{n+2}) - \alpha(h_{n+1} - i_{n+1}) - (\alpha - \beta)(h_n - i_n) = 0$$

$$\therefore\ h_n - i_n = \frac{1}{\sqrt{5\alpha^2 - 4\beta^2}}\left[\left(\frac{\alpha + \sqrt{5\alpha^2 - 4\beta^2}}{2}\right)^n - \left(\frac{\alpha - \sqrt{5\alpha^2 - 4\beta^2}}{2}\right)^n\right].$$

As, however, any variable, such as C_n, may be the sum of a large number of terms from geometrical series, numerical calculation is easier than algebraic. The expressions given by JENNINGS and ROBBINS for the proportion of heterozygotes in the nth generation are wholly independent of linkage. Hence it is clear that by about F_{10} the population contains only 10 percent of *Aa* and as many *Bb* in the homogametic sex, so that equilibrium is nearly reached.

BROTHER-SISTER MATING. AUTOSOMAL GENES

We consider the results of an initial mating $AABB \times aabb$ or reciprocally. The gametic series from an $AB.ab$ ♀ is assumed to be $\alpha AB : \beta Ab : \beta aB : \alpha ab$, from $AB.ab$ ♂, $\gamma AB : \delta Ab : \delta aB : yab$, so that 100β and 100δ are the crossover values. In general these are different, but in mammals β and δ are nearly equal; in the higher insects one of them is zero. There are 100 different types of mating, and owing to the different crossover values in the two sexes, reciprocal crosses do not always yield the same progeny, and therefore the same numbers of matings in the next generation. However, reciprocal crosses occur in the same numbers, and can be grouped together. In the following scheme only one example is given of each type of mating. The total number of types is given in column 2. Thus the following 7 types of mating occur in equal numbers with $AABB \times AAbb$:

$$AAbb \times AABB,\ aaBB \times AABB,\ AABB \times aaBB,\ aabb \times aaBB,$$
$$aaBB \times aabb,\ aabb \times AAbb,\ AAbb \times aabb.$$

In the third column the numbers of each kind of mating in the $(n+1)$th generation are given in terms of similar numbers in the nth. To save space the suffixes of the latter are omitted, for example, H is written for H_n. The method of calculation is similar to that in the sex-linked case. We thus have equations (3.1):

Typical mating	*Number of types*	
$AABB \times AABB$	2	$C_{n+1} = C_n + H + \frac{1}{4}(\alpha^2+\gamma^2)L + \frac{1}{4}(\beta^2+\delta^2)N + \frac{1}{8}Q + \frac{1}{8}R + \frac{1}{8}(\alpha^2+\gamma^2)U + \frac{1}{8}(\beta^2+\delta^2)V + \frac{1}{16}\alpha^2\gamma^2W + \frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{16}\beta^2\delta^2Y.$
$AAbb \times AAbb$	2	$D_{n+1} = D + I + \frac{1}{4}(\alpha^2+\gamma^2)M + \frac{1}{4}(\beta^2+\delta^2)P + \frac{1}{8}Q + \frac{1}{8}S + \frac{1}{8}(\beta^2+\delta^2)U + \frac{1}{8}(\alpha^2+\gamma^2)V + \frac{1}{16}\beta^2\delta^2W + \frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{16}\alpha^2\gamma^2Y.$
$AABB \times aabb$	2	$E_{n+1} = \frac{1}{16}\alpha^2\gamma^2W + \frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{16}\beta^2\delta^2Y.$
$AAbb \times aaBB$	2	$F_{n+1} = \frac{1}{16}\beta^2\delta^2W + \frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{16}\alpha^2\gamma^2Y.$
$AABB \times AAbb$	8	$G_{n+1} = \frac{1}{16}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AABB \times AABb$	8	$H_{n+1} = \frac{1}{2}H + \frac{1}{4}(\alpha\beta+\gamma\delta)(L+N) + \frac{1}{8}R + \frac{1}{16}(\alpha^2+2\alpha\beta+\gamma^2+2\gamma\delta)U + \frac{1}{16}(2\alpha\beta+\beta^2+2\gamma\delta+\delta^2)V + \frac{1}{16}\alpha\gamma(\alpha\delta+\beta\gamma)W + \frac{1}{16}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X + \frac{1}{16}\beta\delta(\alpha\delta+\beta\gamma)Y.$
$AAbb \times AABb$	8	$I_{n+1} = \frac{1}{2}I + \frac{1}{4}(\alpha\beta+\gamma\delta)(M+P) + \frac{1}{8}S + \frac{1}{16}(2\alpha\beta+\beta^2+2\gamma\delta+\delta^2)U + \frac{1}{16}(\alpha^2+2\alpha\beta+y^2+2\gamma\delta)V + \frac{1}{16}\beta\delta(\alpha\delta+\beta\gamma)W + \frac{1}{16}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X + \frac{1}{16}\alpha\gamma(\alpha\delta+\beta\gamma)Y.$
$AABB \times Aabb$	8	$J_{n+1} = \frac{1}{16}(\alpha^2+\gamma^2)U + \frac{1}{16}(\beta^2+\delta^2)V + \frac{1}{16}\alpha\gamma(\alpha\delta+\beta\gamma)W + \frac{1}{16}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X + \frac{1}{16}\beta\delta(\alpha\delta+\beta\gamma)Y.$
$AAbb \times AaBB$	8	$K_{n+1} = \frac{1}{16}(\beta^2+\delta^2)U + \frac{1}{16}(\alpha^2+\gamma^2)V + \frac{1}{16}\beta\delta(\alpha\delta+\beta\gamma)W + \frac{1}{16}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X + \frac{1}{16}\alpha\gamma(\alpha\delta+\beta\gamma)Y.$
$AABB \times AB.ab$	4	$L_{n+1} = \frac{1}{4}(\alpha^2+\gamma^2)L + \frac{1}{4}(\beta^2+\delta^2)N + \frac{1}{8}(\alpha^2+\gamma^2)U + \frac{1}{8}(\beta^2+\delta^2)V + \frac{1}{8}\alpha^2\gamma^2W + \frac{1}{8}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{8}\beta^2\delta^2Y.$
$AAbb \times Ab.aB$	4	$M_{n+1} = \frac{1}{4}(\alpha^2+\gamma^2)M + \frac{1}{4}(\alpha^2+\delta^2)P + \frac{1}{8}(\beta^2+\delta^2)U + \frac{1}{8}(\alpha^2+\gamma^2)V + \frac{1}{8}\beta^2\delta^2W + \frac{1}{8}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{8}\alpha^2\gamma^2Y.$

Typical mating	*Number of types*	
$AABB \times Ab.aB$	4	$N_{n+1} = \frac{1}{8}R + \frac{1}{8}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb \times AB.ab$	4	$P_{n+1} = \frac{1}{8}S + \frac{1}{8}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AABb \times AABb$	4	$Q_{n+1} = 2G + \frac{1}{2}(H+I+J+K) + \frac{1}{4}(\alpha^2+\gamma^2)(L+M) + \frac{1}{4}(\beta^2+\delta^2)(N+P) + \frac{1}{4}Q + \frac{1}{8}(R+S+T) + \frac{1}{8}(\alpha^2+\alpha\beta+\beta^2+\gamma^2+\gamma\delta+\delta^2)(U+V) + \frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y) + \frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb \times AaBB$	4	$R_{n+1} = \frac{1}{4}(\beta^2+\delta^2)L + \frac{1}{4}(\alpha^2+\gamma^2)N + \frac{1}{8}R + \frac{1}{8}(\beta+\delta)U + \frac{1}{8}(\alpha+\gamma)V + \frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y) + \frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb \times Aabb$	4	$S_{n+1} = \frac{1}{4}(\beta^2+\delta^2)M + \frac{1}{4}(\alpha^2+\gamma^2)P + \frac{1}{8}S + \frac{1}{8}(\alpha+\gamma)U + \frac{1}{8}(\beta+\delta)V + \frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y) + \frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb \times aaBb$	4	$T_{n+1} = \frac{1}{8}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y) + \frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb \times AB.ab$	8	$U_{n+1} = \frac{1}{2}J + \frac{1}{4}(\alpha\beta+\gamma\delta)(L+N) + \frac{1}{8}(S+T) + \frac{1}{8}(\alpha+\gamma)U + \frac{1}{8}(\beta+\delta)V + \frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)W + \frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X + \frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)Y.$
$AABb \times Ab.aB$	8	$V_{n+1} = \frac{1}{2}K + \frac{1}{4}(\alpha\beta+\gamma\delta)(M+P) + \frac{1}{8}(R+T) + \frac{1}{8}(\beta+\delta)U + \frac{1}{8}(\alpha+\gamma)V + \frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)W + \frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X + \frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)Y.$
$AB.ab \times AB.ab$	1	$W_{n+1} = 2(E+J) + \frac{1}{2}(\alpha^2+\gamma^2)L + \frac{1}{2}(\beta^2+\delta^2)N + \frac{1}{4}(S+T) + \frac{1}{4}(\alpha^2+\gamma^2)U + \frac{1}{4}(\beta^2+\delta^2)V + \frac{1}{4}\alpha^2\gamma^2W + \frac{1}{4}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{4}\beta^2\delta^2Y.$
$AB.ab \times Ab.aB$	2	$X_{n+1} = \frac{1}{2}T + \frac{1}{2}(\alpha\beta+\gamma\delta)(U+V) + \frac{1}{2}\alpha\beta\gamma\delta(W+2X+Y).$
$Ab.aB \times Ab.aB$	1	$Y_{n+1} = 2(F+K) + \frac{1}{2}(\alpha^2+\gamma^2)M + \frac{1}{2}(\beta^2+\delta^2)P + \frac{1}{4}(R+T) + \frac{1}{4}(\beta^2+\delta^2)U + \frac{1}{4}(\alpha^2+\gamma^2)V + \frac{1}{4}\beta^2\delta^2W + \frac{1}{4}(\alpha^2\delta^2+\beta^2\gamma^2)X + \frac{1}{4}\alpha^2\gamma^2Y.$

Now let $c_n = C_n - D_n$, $d_n = E_n - F_n$, $e_n = H_n - I_n$, $f_n = J_n - K_n$, $g_n = L_n - M_n$, $h_n = N_n - P_n$, $i_n = R_n - S_n$, $j_n = U_n - V_n$, $k_n = W_n - Y_n$.

Hence we have the equations (3.2):

$$c_{n+1} = c_n + e_n + \tfrac{1}{4}(\alpha^2+\gamma^2)g_n + \tfrac{1}{4}(\beta^2+\delta^2)h_n + \tfrac{1}{8}i_n + \tfrac{1}{8}(\alpha-\beta+\gamma-\delta)j_n + \tfrac{1}{16}(\alpha^2\gamma^2-\beta^2\delta^2)k_n.$$

$$d_{n+1} = \tfrac{1}{16}(\alpha^2\gamma^2-\beta^2\delta^2)k_n.$$

$$e_{n+1} = \tfrac{1}{2}e_n + \tfrac{1}{4}(\alpha\beta+\gamma\delta)(g_n+h_n) + \tfrac{1}{8}i_n + \tfrac{1}{16}(\alpha-\beta+\gamma-\delta)j_n + \tfrac{1}{16}(\alpha\gamma-\beta\delta)(\alpha\delta+\beta\gamma)k_n.$$

$$f_{n+1} = \tfrac{1}{16}(\alpha-\beta+\gamma-\delta)j_n + \tfrac{1}{16}(\alpha\gamma-\beta\delta)(\alpha\delta+\beta\gamma)k_n.$$

$$g_{n+1} = \tfrac{1}{4}(\alpha^2+\gamma^2)g_n + \tfrac{1}{4}(\beta^2+\delta^2)h_n + \tfrac{1}{8}(\alpha-\beta+\gamma-\delta)j_n + \tfrac{1}{8}(\alpha^2\gamma^2-\beta^2\delta^2)k_n.$$

$$h_{n+1} = \tfrac{1}{8}i_n.$$

$$i_{n+1} = \tfrac{1}{4}(\beta^2+\delta^2)g_n + \tfrac{1}{4}(\alpha^2+\gamma^2)h_n + \tfrac{1}{8}i_n - \tfrac{1}{8}(\alpha-\beta+\gamma-\delta)j_n.$$

$$j_{n+1} = \tfrac{1}{2}f_n + \tfrac{1}{4}(\alpha\beta+\gamma\delta)(g_n+h_n) - \tfrac{1}{8}i_n + \tfrac{1}{8}(\alpha-\beta+\gamma-\delta)j_n + \tfrac{1}{8}(\alpha\gamma-\beta\delta)(\alpha\delta+\beta\gamma)k_n.$$

$$k_{n+1} = 2(d_n+f_n) + \tfrac{1}{2}(\alpha^2+\gamma^2)g_n + \tfrac{1}{2}(\beta^2+\delta^2)g_n - \tfrac{1}{4}i_n + \tfrac{1}{4}(\alpha-\beta+\gamma-\delta)j_n + \tfrac{1}{4}(\alpha^2\gamma^2-\beta^2\delta^2)k_n.$$

When $n=0$, $E_0=1 \therefore d_0=1$, the other terms being zero.

When $n=\infty$, $C_\infty + D_\infty = 1$, and c_∞ is finite, the other terms being zero.

We now have to find $\xi, \eta, \theta, \kappa, \lambda, \mu, \nu, \phi$ so that:

$$c_{n+1} + \zeta d_{n+1} + \eta e_{n+1} + \theta f_{n+1} + \kappa g_{n+1} + \lambda h_{n+1} + \mu i_{n+1} + \nu j_{n+1} + \phi k_{n+1}$$
$$\equiv c_n + \zeta d_n + \eta e_n + \theta f_n + \kappa g_n + \lambda h_n + \mu i_n + \nu j_n + \phi k_n.$$

The conditions for this to be the case are equations (3.3)

$$\zeta = 2\phi$$
$$\eta = 1 + \tfrac{1}{2}\eta$$
$$\theta = \tfrac{1}{2}\nu + 2\phi$$
$$\kappa = \tfrac{1}{4}(\alpha^2 + \gamma^2)(1 + \kappa + 2\phi) + \tfrac{1}{4}(\beta^2 + \delta^2)\mu + \tfrac{1}{4}(\alpha\beta + \gamma\delta)(\eta + \nu).$$
$$\lambda = \tfrac{1}{4}(\beta^2 + \delta^2)(1 + \kappa + 2\phi) + \tfrac{1}{4}(\alpha^2 + \gamma^2)\mu + \tfrac{1}{4}(\alpha\beta + \gamma\delta)(\eta + \nu).$$
$$\mu = \tfrac{1}{8}(1 + \eta + \lambda + \mu - \nu - 2\phi).$$
$$\nu = \tfrac{1}{16}(\alpha - \beta + \gamma - \delta)(2 + \eta + \theta + 2x - 2\mu + 2\nu + 4\phi).$$
$$\phi = \tfrac{1}{16}(\alpha^2\gamma^2 - \beta^2\delta^2)(1 + \zeta + 2\kappa + 4\phi) + \tfrac{1}{16}(\alpha\gamma - \beta\delta)(\alpha\delta + \beta\gamma)(\eta + \theta + 2\nu).$$

We eliminate $\eta = 2$, and $\phi = \frac{1}{2}\zeta$. We also subtract and add together the fourth and fifth of these equations. We put $x = \frac{1}{2}(\beta + \delta)$, the average crossover value, and also $y = \frac{1}{2}(\alpha\beta + \gamma\delta)$, $z = \alpha\gamma + \beta\delta$, $q = \frac{1}{2} - x$. Hence:

$$2\theta = \nu + 2\zeta.$$
$$\kappa - \lambda = q(1 + \zeta + \kappa - \mu).$$
$$2\kappa + 2\lambda = 1 + y + (1 - y)(\zeta + \kappa) + y\nu.$$
$$7\mu = 3 - \zeta + \lambda - \nu.$$
$$8\nu = q(3 + 2\zeta + \theta + 2\kappa - 2\mu + 2\nu).$$
$$4\zeta = q(3 + \theta + 2\nu) - qz(2 + 3\zeta - \theta + 2\kappa - 2\nu).$$

Omitting some rather tedious algebra, the solution of these equations is:

$$\zeta = \frac{q}{2 - 3q}, \quad \theta = \frac{2q}{2 - 3q}, \quad \kappa = \frac{1}{2 - 3q},$$
$$\lambda = \frac{1 - 2q}{2 - 3q}, \quad \mu = \frac{1 - 2q}{2 - 3q}, \quad \nu = \frac{2q}{2 - 3q}$$

as may easily be verified.

$$\therefore \; c_\infty = c_n + 2e_n + \frac{1}{1 + 6x}[(1 - 2x)(d_n + 2f_n + 2j_n + \tfrac{1}{2}k_n) + 2g_n + 4x(h_n + i_n)] \tag{3.4}$$

and $y = \frac{1}{2}(1 - c_\infty)$.

In the case considered, $d_0 = 1$, $\therefore c_\infty = \zeta d_0 = 1 - 2x/1 + 6x$. Hence the proportion of crossover zygotes, $y = 4x/1 + 6x$ (3.5).

This is plotted in figure 1. If there is 50 percent crossing over in both sexes, $x=\frac{1}{2}$, $y=\frac{1}{2}$. If there is 50 percent in one sex, and none in the other, $x=\frac{1}{4}$, $y=5/12$, that is 5/12 only of the zygotes are crossovers.

To solve the equations (3.1) completely, we require, besides the equations (3.2), a group of 13 equations for C_n+D_n, etc. and also for the symmetrical terms G_n, Q_n, T_n, and X_n. The full expression for D_n is the sum of a constant term with the nth terms of 19 geometrical series. Their ratios are $\frac{1}{2}$ and the irrational roots of two algebraic equations of the 7th and 11th degrees. These equations can, in part at least, be reduced to quartics, but at least one quartic is irreducible. Hence only numerical calculation is practicable.

PARENTS AND OFFSPRING MATING. SEX-LINKED GENES

In this system of mating a father is mated to his own daughter, a son of this union to his mother and subsequently to his daughter, and so on indefinitely. JENNINGS (1917) has dealt with it in the case of unlinked genes. We consider the results of inbreeding where the matings $AABB\times ab$ and $aabb\times AB$ are made in equal numbers and the daughters (assuming the female sex to be homogametic) backcrossed to the fathers. If the sons were backcrossed to their mothers they would of course give 100 percent noncrossover homozygotes at once. The result of the cross considered, between fathers and F_1 daughters, is the same as if the F_1 were crossed with one another, and their children (F_2) backcrossed to parents. It will be shown later that this latter procedure gives the maximum of crossing over of autosomal genes. The case is fairly simple, since many types of mating are impossible after the first generation. For example *aabb* mothers have only *ab* sons. β is the crossover proportion, and $\alpha=1-\beta$.

Typical mating	*Number of types*	
$AABB\times AB$	2	$C_{n+1}=C_n+\frac{1}{2}E_n+\frac{1}{4}\alpha G_n+\frac{1}{4}\beta J_n.$
$AAbb\times Ab$	2	$D_{n+1}=D_n+\frac{1}{2}F_n+\frac{1}{4}\alpha H_n+\frac{1}{4}\beta I_n.$
$AABb\times AB$	4	$E_{n+1}=\frac{1}{2}E_n+\frac{1}{4}F_n+\frac{1}{4}\beta G_n+\frac{1}{4}\alpha J_n.$
$AABb\times Ab$	4	$F_{n+1}=\frac{1}{4}E_n+\frac{1}{2}F_n+\frac{1}{4}\beta H_n+\frac{1}{4}\alpha I_n.$
$AB.ab\times AB$	2	$G_{n+1}=\frac{1}{4}\alpha G_n+\frac{1}{2}\alpha I_n+\frac{1}{4}\beta J_n.$
$Ab.aB\times Ab$	2	$H_{n+1}=\frac{1}{4}\alpha H_n+\frac{1}{4}\beta I_n+\frac{1}{2}\beta J_n.$
$AB.ab\times Ab$	2	$I_{n+1}=\frac{1}{2}\beta(G_n+I_n).$
$Ab.aB\times AB$	2	$J_{n+1}=\frac{1}{2}\beta(H_n+J_n).$

(4.1)

Putting $c_n=C_n-D_n$, $d_n=E_n-F_n$, $e_n=G_n-H_n$, $f_n=I_n-J_n$

$$\therefore\quad \begin{aligned} c_{n+1} &= c_n+\tfrac{1}{2}d_n+\tfrac{1}{4}\alpha e_n-\tfrac{1}{4}\beta f_n \\ d_{n+1} &= \tfrac{1}{4}d_n+\tfrac{1}{4}\beta e_n-\tfrac{1}{4}\alpha f_n \\ e_{n+1} &= \tfrac{3}{4}\alpha e_n+\tfrac{3}{4}(2\alpha-\beta)f_n \\ f_{n+1} &= \tfrac{1}{2}\beta(e_n+f_n) \end{aligned} \qquad (4.2)$$

Put $c_{n+1}+\zeta d_{n+1}+\eta e_{n+1}+\theta f_{n+1} \equiv c_n+\zeta d_n+\eta e_n+\theta f_n$.

$$\left.\begin{aligned} \therefore\ \zeta &= \tfrac{1}{2}+\tfrac{1}{4}\zeta \\ \eta &= \tfrac{1}{4}\alpha+\tfrac{1}{4}\beta\zeta+\tfrac{3}{4}\alpha\eta+\tfrac{1}{2}\beta\theta \\ \theta &= -\tfrac{1}{4}\beta-\tfrac{1}{4}\alpha\zeta+\tfrac{1}{4}(2\alpha-\beta)\eta+\tfrac{1}{2}\beta\theta \end{aligned}\right\} \qquad (4.3)$$

Putting $\beta = x$,

$$\left.\begin{aligned} \zeta &= \tfrac{2}{3},\ \eta = \frac{6-7x}{3(2+3x)},\ \theta = \frac{2-9x}{3(2+3x)} \\ \therefore\ c_\infty &= c_n+\tfrac{2}{3}d_n+\frac{(6-7x)e_n+(2-9x)f_n}{3(2+3x)}. \end{aligned}\right\} \qquad (4.4)$$

In the case considered $e_1 = G_1 = 1$

$$\therefore\ c_\infty = \frac{6-7x}{6+9x}$$

$$\therefore\ y = \frac{8x}{6+9x}. \qquad (4.5)$$

Hence with 50 percent crossing over in the homogametic sex the final proportion of crossover zygotes is 8/21. If both sexes of F_1 are crossed back to the parents,

$$y = \frac{4x}{6+9x}.$$

In this case it is quite possible to solve the equations (4.1) completely. C_n differs from C_∞ by the sum of six terms of geometric series, whose ratios are

$$\frac{1}{4},\ \frac{3}{4},\ \frac{2x \pm \sqrt{9-26x+19x^2}}{4},\ \frac{2x \pm \sqrt{9-26x+23x^2}}{4}.$$

Even here however the expression is rather complicated.

PARENT AND OFFSPRING MATING. AUTOSOMAL GENES

The mating system is the same as in the last case, except that both sons and daughters in F_1 are crossed back to the parents. This case has been considered, for unlinked genes, by both JENNINGS (1917) and WRIGHT (1921b). If 100β and 100δ are the crossover percentages, and $\alpha+\beta = \gamma+\delta = 1$, we arrive at equations (5.1).

Typical mating	*Number of types*	
AABB×AABB	2	$C_{n+1}=C+F+\frac{1}{4}(\alpha+\gamma)J+\frac{1}{4}(\beta+\delta)L.$
AAbb×AAbb	2	$D_{n+1}=D+G+\frac{1}{4}(\alpha+\gamma)K+\frac{1}{4}(\beta+\delta)M.$
AABb×AABb	4	$E_{n+1}=\frac{1}{2}(E+F+G)+\frac{1}{4}(H+I+N+P).$
AABB×AABb	8	$F_{n+1}=\frac{1}{8}E+\frac{1}{2}F+\frac{1}{8}H+\frac{1}{4}(\beta+\delta)J+\frac{1}{4}(\alpha+\gamma)L+\frac{1}{16}(\alpha+\gamma)N+\frac{1}{16}(\beta+\delta)P.$
AAbb×AABb	8	$G_{n+1}=\frac{1}{8}E+\frac{1}{2}G+\frac{1}{8}I+\frac{1}{4}(\beta+\delta)K+\frac{1}{4}(\alpha+\gamma)M+\frac{1}{16}(\beta+\delta)N+\frac{1}{16}(\alpha+\gamma)P.$
AABb×AaBB	4	$H_{n+1}=\frac{1}{4}H+\frac{1}{8}(\beta+\delta)N+\frac{1}{8}(\alpha+\gamma)P.$
AABb×Aabb	4	$I_{n+1}=\frac{1}{4}I+\frac{1}{8}(\alpha+\gamma)N+\frac{1}{8}(\beta+\delta)P.$
AABB×AB.ab	4	$J_{n+1}=\frac{1}{4}(\alpha+\gamma)J+\frac{1}{8}(\beta+\delta)L+\frac{1}{8}(\alpha+\gamma)N+\frac{1}{8}\alpha\gamma Q+\frac{1}{16}(\alpha\delta+\beta\gamma)R.$
AAbb×Ab.aB	4	$K_{n+1}=\frac{1}{4}(\alpha+\gamma)K+\frac{1}{8}(\beta+\delta)M+\frac{1}{8}(\alpha+\gamma)P+\frac{1}{8}\alpha\gamma S+\frac{1}{16}(\alpha\delta+\beta\gamma)R.$
AABB×Ab.aB	4	$L_{n+1}=\frac{1}{8}(\beta+\delta)(L+P)+\frac{1}{16}(\alpha\delta+\beta\gamma)R+\frac{1}{8}\beta\delta S.$
AAbb×AB.ab	4	$M_{n+1}=\frac{1}{8}(\beta+\delta)(M+N)+\frac{1}{16}(\alpha\delta+\beta\gamma)R+\frac{1}{8}\beta\delta Q.$
AABb×AB.ab	8	$N_{n+1}=\frac{1}{8}I+\frac{1}{8}(\beta+\delta)J+\frac{1}{8}(\alpha+\gamma)M+\frac{1}{16}(4+\alpha+\gamma)N+\frac{1}{16}(\beta+\delta)P+\frac{1}{8}(\alpha\delta+\beta\gamma)Q+\frac{1}{16}(\alpha\gamma+\beta\delta)R.$
AABb×Ab.aB	8	$P_{n+1}=\frac{1}{8}H+\frac{1}{8}(\beta+\delta)K+\frac{1}{8}(\alpha+\gamma)L+\frac{1}{16}(4+\alpha+\gamma)P+\frac{1}{16}(\beta+\delta)N+\frac{1}{8}(\alpha\delta+\beta\gamma)S+\frac{1}{16}(\alpha\gamma+\beta\delta)R.$
AB.ab×AB.ab	1	$Q_{n+1}=\frac{1}{4}(\alpha+\gamma)(J+N)+\frac{1}{2}\alpha\gamma Q+\frac{1}{4}(\alpha\delta+\beta\gamma)R.$
AB.ab×Ab.aB	2	$R_{n+1}=\frac{1}{4}(\beta+\delta)(L+M+N+P)+\frac{1}{2}\beta\delta(Q+S)+\frac{1}{4}(\alpha\delta+\beta\gamma)R.$
Ab.aB×Ab.aB	1	$S_{n+1}=\frac{1}{2}(\alpha+\gamma)(K+P)+\frac{1}{2}\alpha\gamma S+\frac{1}{4}(\alpha\delta+\beta\gamma)R.$

Let $c_n=C_n-D_n$, $d_n=F_n-G_n$, $e_n=H_n-I_n$, $f_n=J_n-K_n$, $g_n=L_n-M_n$, $h_n=N_n-P_n$, $i_n=Q_n-S_n$. Let $\beta+\delta=2x$, $\beta\delta=p$.

$$\left.\begin{aligned}
\therefore\ c_{n+1} &= c_n + d_n + \tfrac{1}{2}(1-x)f_n + \tfrac{1}{2}xg_n.\\
d_{n+1} &= \tfrac{1}{2}d_n + \tfrac{1}{8}e_n + \tfrac{1}{4}xf_n + \tfrac{1}{4}(1-x)g_n + \tfrac{1}{8}(1-2x)h_n.\\
e_{n+1} &= \tfrac{1}{4}e_n - \tfrac{1}{4}(1-2x)h_n.\\
f_{n+1} &= \tfrac{1}{2}(1-x)f_n + \tfrac{1}{4}xg_n + \tfrac{1}{4}(1-x)h_n + \tfrac{1}{8}(1-2x+p)i_n.\\
g_{n+1} &= \tfrac{1}{4}x(g_n-h_n) - \tfrac{1}{8}pi_n.\\
h_{n+1} &= -\tfrac{1}{8}e_n + \tfrac{1}{4}xf_n - \tfrac{1}{4}(1-x)g_n + \tfrac{1}{8}(3-2x)h_n + \tfrac{1}{4}(x-p)i_n.\\
i_{n+1} &= (1-x)(f_n+h_n) + \tfrac{1}{2}(1-2x+p)i_n.
\end{aligned}\right\} \quad (5.2)$$

In order to have $c_n+\zeta d_n+\eta e_n+\theta f_n+\kappa g_n+\lambda h_n+\mu i_n$ independent of n, it is necessary that:

$$\left.\begin{aligned}
\zeta &= 1 + \tfrac{1}{2}\zeta.\\
\eta &= \tfrac{1}{8}\zeta + \tfrac{1}{4}\eta - \tfrac{1}{8}\lambda.\\
\theta &= \tfrac{1}{2}(1-x) + \tfrac{1}{4}x\zeta + \tfrac{1}{2}(1-x)\theta + \tfrac{1}{4}x\lambda + (1-x)\mu.\\
\kappa &= \tfrac{1}{2}x + \tfrac{1}{4}(1-x)\zeta + \tfrac{1}{4}x(\theta+\kappa) - \tfrac{1}{4}(1-x)\lambda.\\
\lambda &= \tfrac{1}{8}(1-2x)\zeta - \tfrac{1}{4}(1-2x)\eta + \tfrac{1}{4}(1-x)\theta - \tfrac{1}{4}x\kappa + \tfrac{1}{8}(3-2x)\lambda + (1-x)\mu.\\
\mu &= \tfrac{1}{8}(1-2x+p)\theta - \tfrac{1}{8}p\kappa + \tfrac{1}{4}(x-p)\lambda + \tfrac{1}{2}(1-2x+p)\mu.
\end{aligned}\right\} \quad (5.3)$$

Hence $\zeta = 2, \eta = \frac{2x}{1+4x}, \theta = \frac{2}{1+4x}, \kappa = \frac{4x}{1+4x}, \lambda = \frac{2(1-2x)}{1+4x}$,

$$\mu = \frac{1-2x}{1+4x},$$

as may readily be verified.

$$\therefore \quad c_\infty = c_n + 2d_n + \frac{1}{1+4x}[2f_n + 2x(e_n + 2g_n) + (1-2x)(2h_n + i_n)] \quad (5.4)$$

In the case considered $J_0 = f_0 = \frac{1}{2} \therefore c_\infty = 1/1+4x$

$$\therefore \quad y = \frac{2x}{1+4x}. \quad (5.5)$$

Hence with 50 percent crossing over in both sexes, $y = \frac{1}{3}$, with 50 percent crossing over in one only, $y = \frac{1}{4}$. In order to obtain the maximum proportion of crossovers, we should mate F_1 *inter se*, and then mate F_2 back to F_1 of the opposite sex. In this case

$$Q_1 = i_1 = 2, \text{ so } c_\infty = \frac{1-2x}{1+4x}, \text{ and } y = \frac{3x}{1+4x}.$$

Hence when $x = \frac{1}{2}$, $y = \frac{1}{2}$, and with 50 percent crossing over in one sex only, $y = \frac{3}{8}$. The approach to equilibrium involves the solution of equations (5.2) and a set of nine similar equations for $C_n + D_n$, etc. along with E_n and R_n. C_n differs from C_∞ by the sum of terms from 14 geometric series.

INBREEDING WITH ANY INITIAL POPULATION

The five systems of equations (1.1), (2.1), (3.1), (4.1) and (5.1) are true whatever be the initial composition of the population, provided that it contains the genes *A*, *a*, *B*, and *b* in equal numbers. This is not in general so. But we can render any population symmetrical by adding to it three other suitably chosen populations, these latter being added after one generation of inbreeding, so as not to mate with the group first considered. This addition does not affect the proportion of crossover zygotes, and the proportion of the genes is of course unaltered by inbreeding.

An example will make the method clear. We desire to know the final fate of a population consisting of zygotes in the proportion 1*AABB*:4*aaBb*, when mating is at random for one generation, and afterwards brothers and sisters only are mated. After one generation we add to it equal numbers of the children of three populations consisting of (1*AABB*:4*Aabb*), (1*aabb*:

$4AABb$) and ($1aabb$:$4AaBB$). Hence, out of 100 typical matings of the mixed F_1, 2 are $AABB \times AABB$, 2 $aabb \times aabb$, 4 of each of the 8 types exemplified by $AABB \times aaBb$, 16 of each of the 4 types exemplified by $AABb \times AABb$. Dividing by 50, to give a total of 2:

$$c_1 = C_1 = \frac{1}{25};\ f_1 = J_1 = \frac{2}{25};\ Q_1 = \frac{8}{25}.$$

FIGURE 1.—The curves show the percentage of crossover homozygotes at equilibrium (100y), plotted as ordinates against the average crossover value (100x) as abscissa, for the following five systems of mating:

1. Self-fertilization.
2. Brother-sister mating. Sex-linked genes.
3. Brother-sister mating. Autosomal genes.
4. Parent-offspring mating, F_1 daughters backcrossed to father, sex-linked genes.
5. Parent-offspring mating, both F_1 sons and daughters backcrossed to parents, autosomal genes.

Hence by equation (3.4), $c_\infty = 1/25 + 2/25 \cdot 2(1-2x)/1+6x$.

$$\therefore\ y = \frac{10 + 76x}{25(1 + 6x)}.$$

This is the proportion of crossover zygotes. In the population first considered the ratio $A:a$ is 1:4, that of $B:b$ is 3:2. Hence if $[AABB]$ is the proportion of that type in the final population,

$$[AABB] + [AAbb] = \frac{1}{5},\ [AABB] + [aaBB] = \frac{3}{5},\ [AAbb] + [aaBB] = y$$

$$\therefore\ [AABB] = \frac{5 + 22x}{25(1 + 6x)},\ [AAbb] = \frac{8x}{25(1 + 6x)},$$

$$[aaBB] = \frac{10 + 68x}{25(1 + 6x)},\quad [aabb] = \frac{10 + 52x}{25(1 + 6x)}.$$

Similarly the final composition of any other population may be found. It is often more convenient to split it up into groups, and consider each separately. Thus in the above case 1/25 of the final population is derived from $AABB \times AABB$, that is, is all $AABB$; 16/25 is derived from the mating $aaBb \times aaBb$, that is, consists of equal numbers of $aaBB$ and $aabb$; 8/25 is derived from $AABb \times aaBb$, and the reciprocal cross. This gives all four zygotic types in proportions depending on the value of x. It is quite possible to form equations for the results of breeding from a population containing arbitrary numbers of each of the 20 zygotic types, but the expressions obtained are neither short nor elegant.

DOUBLE CROSSING OVER

Consider the inbreeding of zygotes ABC/abc, where the 3 genes A, B, C are linked and in that order. Let p be the proportion of crossing over between AB (that is, 100p the crossover value), and let q, r be the same proportions for BC, AC. Then the proportion of double crossovers is obviously $\frac{1}{2}(p+q-r)$. Similarly if, after inbreeding, the proportions of crossover zygotes are f(p), f(q), f(r), the proportion of double crossover zygotes, that is, $aaBBcc$ and $AAbbCC$, is $\frac{1}{2}[f(p)+f(q)-f(r)]$. Now f(p) is always of the form kp/1+lp. Hence we have, for the proportion Z of double crossovers,

$$2Z = \frac{kp}{1 + lp} + \frac{kq}{1 + lq} + \frac{kr}{1 + lr}$$

$$\therefore\ Z = \frac{k(p + q - r + 2lpq + l^2pqr)}{2(1 + lp)(1 + lq)(1 + lr)}.$$

Now $\frac{1}{2}(p+q-r)$ is the proportion of gametic double crossovers, and therefore smaller than pq, owing to interference, unless p and q are quite large. Hence since $l>1$ the last two terms in the nominator are the most important. When p and q are small, r is practically equal to their sum, and $Z = klpq$, approximately.

That is to say whereas the proportion of single gametic crossovers is k times the corresponding gametic value when the latter is small, the proportion of zygotic double crossovers is kl times the proportion of gametic double crossovers calculated if there were no interference, and many more times the real value. The values of k and kl, collected from equations (1.3), (2.5), (3.5), (4.5), (5.5) and their modifications, are given in table 2.

TABLE 2

TYPE OF INBREEDING	TYPE OF LINKAGE	k	kl
Self-fertilization	Autosomal	2	4
Brother-sister mating	Autosomal	4	24
Brother-sister mating	Sex-linked	8/3	32/3
Parent-offspring mating	Autosomal	2	8
Parent-offspring mating after F_1	Autosomal	3	12
Parent-offspring mating both ways	Sex-linked	2/3	1
Parent-offspring mating father-daughter, or after F_1	Sex-linked	4/3	2

DISCUSSION

The method of calculation here employed cannot be applied to most other types of inbreeding. It is wholly inapplicable to the important case where a male is bred to a large group of his half-sisters in each generation. The case of double first cousin matings with autosomal linkage involves the consideration of 10,000 different pairs of mating types, and other systems are still more complex. It may prove possible to solve such problems by an extension of WRIGHT'S (1921a) correlation method, but we have been unable to do so.

Inbreeding may be undertaken for several purposes. It may be desired to obtain and fix as many types as possible after crossing two different varieties, to obtain a pure line of a desired type, or merely a pure line of any sort. In each case it is desirable to encourage crossing over. If the genetics of the organism concerned are known already, this can doubtless be done by *ad hoc* matings. In general this is not possible. The reason why crossing over, that is, recombination, is desirable in all types of inbreeding is as follows. Hybrid vigor may be due partly to heterozygosis as such, but it is probably also in part due to the presence in the hybrid of dominant

genes contributed by the two parents. If so the vigor of an extracted pure line will depend on the numbers of such dominants which are combined in it. In so far as they are linked, the probability of such a combination will depend on the type of mating practised.

It is clear from table 2 that the differences are very considerable. For example a recombination involving three fairly closely linked genes is about 3 times as likely with brother-sister mating as with parent-offspring mating, and six times as likely as with self-fertilization. In the case of sex-linked genes the probability is over 10 times as great with brother-sister mating as with parent-offspring mating. However the disadvantages of the latter are considerably diminished if instead of beginning it by crossing the F_1 to the parents, the first parent-offspring mating is between F_1 and F_2. If the ideal genotype requires the occurrence of a number of crossings over the differences between different mating systems are greatly increased.

For example if two pure lines of Drosophila whose males are of composition $Ab(CdE/CdE)(FgH/FgH)$, and $aB(cDe/cDe)(fGh/fGh)$, respectively, are crossed, the proportion of pure lines finally containing all the dominants is 0.98 percent with brother-sister mating, 0.0372 percent with unrestricted parent-offspring mating, even when all crossover values are 50 percent.

It is clear then that the proportion of homozygosis reached is not the sole possible criterion of intensity of inbreeding. But the difference between different systems due to the considerations here outlined will be far more intense in an organism with high linkage, such as Apotettix, Lebistes, Drosophila, or Funaria, than in one with little linkage, such as a mammal or *Triticum vulgare*.

SUMMARY

Formulae are given for the amount of crossing over which is found in the final population when organisms heterozygous for linked genes are inbred according to various systems.

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THE FREQUENCIES OF HOMOZYGOSIS OF FACTORS IN ATTACHED-X FEMALES OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The first evidence that crossing over in *Drosophila melanogaster* occurs at a stage where each chromosome is divided or split into two strands was presented by BRIDGES (1916) in his interpretation of the origin of the rare "equational exceptions," that is, daughters which received two X chromosomes from their mother but were homozygous for a recessive gene carried in a heterozygous condition by their mother. Later L. V. MORGAN (1925), ANDERSON (1925), and STURTEVANT (in press) in their studies on crossing over in attached-X lines, ANDERSON (1929) by his determination of the genotypic constitution of exceptional daughters from a high non-disjunction line, and BRIDGES and ANDERSON (1925) in their analysis of crossing over in the X chromosomes of triploid Drosophila conclusively proved that crossing over in the X chromosome usually, if not always, takes place at a time when each conjugant chromosome is double. REDFIELD (1930) shows that in the third chromosomes of triploid Drosophila crossing over likewise takes place at a "double strand" stage. Data by DOBZHANSKY and STURTEVANT (in press) from their work on II-III translocations lead to a similar conclusion for the second chromosome. Nothing is known regarding the small fourth chromosome since crossovers rarely, if ever, are found.

ANDERSON (1925) studied crossing over between attached-X chromosomes heterozygous for the recessive genes cut (c_t), tan (t), garnet (g), and forked (f). He found that the frequency with which a recessive gene, heterozygous in the mother, appeared in a homozygous condition in the daugh-

ters was a function of its distance from the right or spindle-fiber end of the chromosome. Genes, such as forked, situated near the spindle fiber so that no, or little, double crossing over occurred between their loci and the fiber end, had a frequency of homozygosis equal to one-half their map distance from the attachment point. The progressive increase in homozygosis for factors located further to the left approached a value of 16.7 percent for the two left-most genes involved in his experiments, namely cut (c_t) and tan (t) for which he found percentages of 15.5 and 16.1 respectively. (If the left ends of the attached-X chromosomes assort at random with respect to the spindle fibers, 1 of the 6 possible combinations of the 4 strands, or 16.7 percent, will be homozygous for any recessive factor carried in the distal end.) His detailed analysis of the association of crossover and non-crossover strands for the different regions, derived from determining the genotypic constitution of exceptional daughters, showed that the assortment of strands approached that based on random expectation for the left-most, cut-tan, region involved.

It might be inferred from ANDERSON'S results that for genes located near the left or distal end of the X chromosome the frequency with which they appeared homozygous would closely approach 16.7 percent. But L. V. MORGAN (1925) found for the gene yellow (y), situated at the extreme left end, a frequency of 19.0 percent and for echinus (e_c) 5.5 map-units to the right of yellow, a frequency of 17.3 percent. Her numbers are, however, small and the excess of equationals is not statistically significant. STURTEVANT (in press) found a value of 17.1 percent for scute (s_c) in a total of approximately 25,000 flies. These apparently high values for y, s_c, and e_c indicated the possibility that the assortment or distribution of genes in the left end of the X chromosome was not perfectly random, and at the suggestion of Doctors A. H. STURTEVANT and E. G. ANDERSON an experiment was undertaken to definitely determine whether the frequency of homozygosis for the left end was in excess of 16.7 percent, or that expected on random assortment. I wish to express my sincere appreciation to both of them for their interest and advice and to Doctor STURTEVANT for supplying stocks with which to begin the experiment.

SYNTHESIS OF ATTACHED-X LINE

Females with attached-X's homozygous for the recessive gene forked (f) were made homozygous for a third chromosome recessive factor found by GOWEN (1922), called U.C.-G., which prevents or inhibits crossing over in all chromosomes and causes an approximately random distribution of the entire chromosome complement at the reduction division, and were

mated to yellow^2 (y) males. Among the offspring of this cross several triploid females were obtained which carried one pair of attached-X's homozygous for forked and a free X-chromosome with the factor y. (Doctor STURTEVANT made these preliminary crosses.) These triploid females were mated to scute (s_c), apricot (w^a), echinus (e_c), ruby (r_b), not-forked males and the ensuing triploid daughters mated in individual cultures to yellow males. In the following generation, triploid daughters were mated to s_c, w^a, e_c, r_b, males and this alternating of breeding triploid females carrying attached-X's to the two classes of males continued until through the proper crossovers diploid females were obtained whose attached X-chromosomes were heterozygous for the recessive genes, y, s_c, w^a, e_c, r_b. It was necessary to test each diploid daughter individually in order to determine its genotypic constitution. Two forked females were found which gave equationals for the 5 genes. The forked daughters from these females were used in perpetuating the attached-X line. The map positions of the 5 genes are as follows:

y—0.0
s_c—0.+
w^a—1.5
e_c—5.5
r_b—7.5

No crossing over has ever been observed between y and s_c except in triploids. An attempt was made to introduce the normal allelomorph of forked into the attached-X line to give a check upon the normality of the right end of the attached pair, but due to the difficulty of introducing factors located so near the attachment point this plan was abandoned and the analysis begun with the 5 factors situated in the left end. Fortunately several reversions of forked to its wild-type allelomorph occurred in the attached-X line and it was possible to determine the frequency of homozygosis for this gene. All 5 of the mutant factors used are easily and accurately classified and have relatively slight effect upon viability. Where possible, as in the cases of the yellow and scute loci where yellow^2 and scute^1 were used, the more viable members of the allelomorphic series of genes were chosen.

DATA FROM ATTACHED-X FEMALES

The genotypic constitution of the attached-X females, which were finally secured, was $yf/s_cw^ae_cr_bf$. Since y and s_c may be considered as occupying the same locus there are 8 (2^3) possible combinations of the 4 different loci and these were all found during the course of the analysis and extensive data were obtained for 6 of the 8 types.

Forked females, heterozygous for the 5 mutant genes, were mated to Bar (B) or Beadex (B_x) males in individual culture bottles. Several stocks of Bar and Beadex males were used and the matings with different stocks arranged to avoid inbreeding and secure heterosis. In some cases the parental flies were transferred to fresh culture bottles after 7 days and a second progeny obtained. Without exception, counts were extended through a 9 or 10 day period in order to include as nearly as possible all the offspring from a given mating.

The 8 possible combinations of the 5 factors are:

1. $\dfrac{y \qquad\qquad f}{s_c\ w^a\ e_c\ r_b \quad f}$

2. $\dfrac{s_c \qquad\qquad f}{y\ w^a\ e_c\ r_b \quad f}$

3. $\dfrac{y\ w^a \qquad\qquad f}{s_c \quad e_c\ r_b \quad f}$

4. $\dfrac{y\ w^a\ e_c \qquad f}{s_c \quad r_b \quad f}$

5. $\dfrac{y \qquad r_b \quad f}{s_c\ w^a \quad e_c \quad f}$

6. $\dfrac{y \quad e_c\ r_b \quad f}{s_c\ w^a \qquad\quad f}$

7. $\dfrac{y\ w^a \quad r_b \quad f}{s_c \quad e_c \quad f}$

8. $\dfrac{y \quad e_c \quad f}{s_c\ w^a \quad r_b \quad f}$

The data from these combinations are presented in tables 2 to 9. The frequencies of homozygosis for the mutant genes, found by grouping together all flies from mothers of the same genotypic constitution excepting the forked locus, in the various combinations, are presented in table 1. The

TABLE 1

Percentages of flies equational for $y\ s_c w^a e_c r_b$. Data from females homozygous and heterozygous for forked are combined and given in percent.

COMBINATION	y PERCENT	e_c PERCENT	w^a PERCENT	e_c PERCENT	r_b PERCENT	NUMBER OF FLIES
$\dfrac{y}{s_c\ w^a \quad e_c\ r_b}$	19.9	16.8	16.6	16.6	16.6	14,198
$\dfrac{s_c}{y\ w^a \quad e_c\ r_b}$	17.1	18.8	16.9	16.6	16.5	4,399
$\dfrac{y \qquad r_b}{s_c\ w^a \quad e_c}$	19.5	18.1	17.8	17.8	19.3	10,133
$\dfrac{y \quad e_c}{s_c\ w^a \quad r_b}$	19.4	18.6	18.6	18.7	18.4	3,953
$\dfrac{y \quad e_c\ r_b}{s_c\ w^a}$	18.0	18.9	19.0	17.2	17.5	4,157
$\dfrac{y\ w^a \quad e_c}{s_c \quad r_b}$	18.8	18.7	18.9	18.7	18.3	1,669
$\dfrac{y}{s_c}$	19.3	18.1				3,467

total number of flies obtained in 2 of the classes was so small that it was considered meaningless to determine the frequencies of homozygosis for the different loci and they have been omitted from the table. However, the percentages for y and s_c from females heterozygous for no other mutant factor have been included.

INTERPRETATION AND DISCUSSION OF DATA

These data show conclusively that for genes situated in the left end of the X chromosome the frequency of homozygosis is greater than that expected on random assortment of strands with respect to their fiber attachment. The average frequency for y in 40,306 flies, calculated from 6 different combinations in each of which at least 3,400 flies were counted, is 18.9 percent, a deviation from 16.7 which is statistically significant, the Dev./P.E. = 18.3.

The average value for s_c, determined in the same manner, is less, being 18.2 percent as compared with 18.9 percent for y. That this difference is undoubtedly due to the lesser viability of scute flies is substantiated by the frequencies of 19.3 and 18.1 percent for y and s_c, respectively, among the progeny from females heterozygous for only these 2 factors. For the s_c locus the excess above the theoretical expectation is also significant, the Dev./P.E. = 12.5.

The average percentages for w^a, e_c, and r_b, from the first 5 classes in table 1, are 17.8, 17.4, and 17.7, respectively. The average frequency of homozygosis for these genes is only slightly less than for y and s_c but the differences may be attributed to the fact that they occupy a somewhat closer position in the chromosome to the spindle fiber, and the data of MORGAN (1925), ANDERSON (1925 and 1929), and STURTEVANT show a decreasing frequency in homozygosis for genes lying progressively nearer the right end of the X chromosome.

The data show clearly that in the progeny of females carrying the mutant genes in an unbalanced condition, that is, only 1 mutant gene in one of the attached chromosomes and 4 mutant genes in the other, there is a decided and significant excess of females equational for the single gene. For example, the value for y in the combination ($y/s_c w^a\ e_c r_b$) was 19.9 percent as contrasted with 16.8 percent for s_c and slightly lower percentages for the other loci. The high value for y is in part accounted for by the comparatively low frequency of the contrary class of equationals. The corrected value is 19.3 percent. And in the arrangement ($yw^a\ e_c r_b/s_c$) there was a greater frequency of flies equational for s_c than for y or the other

factors even though s_c has a more adverse effect upon viability than any of the other 4 genes. Their cumulative effect, however, was sufficient to appreciably decrease their frequencies of homozygosis.

The frequencies obtained in 5 of the balanced arrangements, that is, 2 genes in one chromosome and 3 in the other, are more uniform but the data show that flies homozygous for any 2 of the 5 mutant genes are more viable than flies equational for the remaining 3. The frequencies calculated from ($yw^a/s_c\ e_c r_b$) mothers do not agree with this generalization but the total number of flies is small and the deviations may well be due to errors in sampling.

If, as the data suggest, an increase in the number of mutant characters lowers the viability it might be expected that the frequency of equationals derived from females heterozygous for an increased number of genes would be less than in these experiments. STURTEVANT, working with attached X's heterozygous for $s_c\ e_c\ c_v\ c_t\ v\ g\ f$, found for s_c, the left-most factor, a value of only 17.1 percent homozygosis.

It is impossible from these data to arrive at any definite conclusion regarding the crossing over mechanism that results in percentages of equationals greater than 16.7 but there are at least two plausible explanations, which need not be elaborated in the absence of complete data. If either the frequency of multiple crossovers is not sufficiently large or there be a disproportionate number of reciprocal as compared with diagonal crossovers, there will be a deviation from the expected percentages based on random assortment in the direction observed in these experiments. The answer awaits the analyses of the genotypic constitutions of daughters from mothers heterozygous for genes distributed along the length of the chromosome.

FORKED REVERSIONS

The attached-X line with which the experiment was begun was homozygous for the recessive gene forked. During the course of the investigations a total of 5 separate reversions of forked to its wild-type allelomorph were found. That only a single reversion occurred in any one culture suggests that they took place during or following the maturation divisions. Reversions of forked to its wild-type allelomorph are not frequent as MORGAN (1929) reported the first occurrence of such. Recently MULLER (1930) succeeded in inducing forked reversions through the agency of X-rays although he failed to find any reversions in his controls.

Since the reversions affected only one of the chromosomes in the attached-X's it was possible to determine the frequency of flies equational

for the forked locus. The data from females heterozygous for the 6 genes are presented in tables 11 to 15. The combined data give a frequency of 4.9 percent of forked equationals among 3,665 flies, a value which closely agrees with those of 5.1 and 5.2 percent reported by STURTEVANT and ANDERSON. The frequencies of the other mutant genes are of the same order as from flies homozygous for forked. This strongly suggests the normality of crossing over in the right ends of the attached chromosomes used.

DETACHMENTS

In addition to the 41,975 exceptional females there occurred 39 regular offspring, which arose through a detachment of the attached-X's. Sixteen regular females and 13 regular males were found, where equal numbers of the 2 sexes are expected. That in some cases detachments take place in oogonial cells, prior to the maturation divisions, is suggested by the occurrence of more than one regular offspring in some of the cultures, several bottles containing 2 and one culture giving rise to 3 regular individuals. The frequency of regular individuals in the progeny of attached-X females is 1 in approximately 1450 flies. ANDERSON (1925) found the proportion of regular offspring to be 1 in about 1300 flies. Four triploid females and 1 male-type intersex arose during the course of the experiment.

SUMMARY

1. Among the progeny from attached-X females heterozygous for the 5 genes, y, s_c, w^a, e_c, r_b, situated in the left end of the X chromosome of *Drosophila melanogaster*, there was a significant excess over the expected 16.7 percent of flies equational for these genes, that is, the segregation of the left ends of the four strands is not at random with respect to the fiber attachment. Counts were made on approximately 42,000 flies.

2. The data as obtained still show some effects of differential viability, although the most viable available mutant allelomorphs were used in each case.

3. Five reversions of forked to its wild-type allelomorph occurred in attached-X females, making it possible to determine the frequency of homozygosis for this gene. It was found to be 4.9 percent.

4. There were 39 regular offspring among the exceptional flies. They arose through detachments of the attached-X's. Four triploid females and 1 male-type intersex were found.

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TABLE 2

Exceptional daughters from attached-X mothers of $\frac{y \qquad\qquad f}{s_c\ w^a\ \ e_e\ r_b\ \ f}$ *constitution.*

f	*y* *f*	*s*$_c$ *w*^{a} *e*$_e$ *r*$_b$ *f*	*s*$_c$ *w*^{a} *e*$_e$ *f*	*s*$_c$ *w*^{a} *f*	*s*$_c$ *f*	*w*^{a} *e*$_e$ *r*$_b$ *f*	*e*$_e$ *r*$_b$ *f*	*r*$_b$ *f*	*s*$_c$ *r*$_b$ *f*	*s*$_c$ *e*$_e$ *r*$_b$ *f*	*y* *r*$_b$ *f*	*w*^{a} *e*$_e$ *f*	*s*$_c$ *w*^{a} *e*$_e$ *r*$_b$	Total
8477	2793	1903	97	189	171	148	192	82	1	2	1	2	1	14,059

TABLE 3

Exceptional daughters from attached-X mothers of $\frac{y\ w^a\ \ e_e\ r_b\ \ f}{s_c \qquad\qquad f}$ *constitution.*

f	*y* *w*^{a} *e*$_e$ *r*$_b$ *f*	*s*$_c$ *f*	*y* *w*^{a} *e*$_e$ *f*	*y* *w*^{a} *f*	*y* *f*	*w*^{a} *e*$_e$ *r*$_b$ *f*	*e*$_e$ *r*$_b$ *f*	*r*$_b$ *f*	*s*$_c$ *r*$_b$ *f*	Total
2704	607	825	26	73	47	36	59	21	1	4399

TABLE 4

Exceptional daughters from attached-X mothers of $\frac{y\ w^a \qquad\qquad f}{s_e \quad e_e\ r_b \quad f}$ *constitution.*

f	*y* *w*ᵃ *f*	*s*ₑ *e*ₑ *r*ᵦ *f*	*y* *f*	*w*ᵃ *f*	*s*ₑ *e*ₑ *f*	*s*ₑ *f*	*e*ₑ *r*ᵦ *f*	*r*ᵦ *f*	*e*ₑ *f*	Total
215	74	59	4	7	2	20	16	1	1	399

TABLE 5

Exceptional daughters from attached-X mothers of $\frac{y\ w^a \quad e_e \quad f}{s_e \quad r_b \quad f}$ *constitution.*

f	*y* *w*ᵃ *e*ₑ	*s*ₑ *r*ᵦ *f*	*y* *w*ᵃ *f*	*y* *f*	*w*ᵃ *e*ₑ *f*	*e*ₑ *f*	*s*ₑ *f*	*r*ᵦ *f*	Total
646	173	195	20	19	15	15	45	36	1164

TABLE 6

Exceptional daughters from attached-X mothers of $\frac{y \qquad r_b \quad f}{s_e\ w^a \quad e_e \quad f}$ *constitution.*

f	*y* *r*ᵦ *f*	*s*ₑ *w*ᵃ *e*ₑ *f*	*y* *f*	*r*ᵦ *f*	*s*ₑ *w*ᵃ *f*	*s*ₑ *f*	*w*ᵃ *e*ₑ *f*	*e*ₑ *f*	*w*ᵃ *f*	+	Total
4607	1291	1237	332	308	107	136	115	113	2	2	8250

TABLE 7

Exceptional daughters from attached-X mothers of $\frac{y \quad e_e\ r_b \quad f}{s_e\ w^a \qquad f}$ *constitution.*

f	*y* *e*ₑ *r*ᵦ *f*	*s*ₑ *w*ᵃ *f*	*y* *e*ₑ *f*	*y* *f*	*e*ₑ *r*ᵦ *f*	*r*ᵦ *f*	*s*ₑ *f*	*w*ᵃ *f*	*e*ₑ *f*	+	Total
1888	451	536	17	109	78	20	39	41	1		1 3181

TABLE 8

Exceptional daughters from attached-X mothers of $\frac{y \quad e_e \quad . \quad f}{s_e \; w^a \quad r_b \quad f}$ *constitution.*

f	*y* *e*$_e$ *f*	*s*$_e$ *w*^{a} *r*$_b$ *f*	*y* *f*	*e*$_e$ *f*	*s*$_e$ *w*^{a} *f*	*s*$_e$ *f*	*w*^{a} *r*$_b$ *f*	*r*$_b$ *f*	Total
2098	596	547	135	109	107	53	56	90	3791

TABLE 9

Exceptional daughters from attached-X mothers of $\frac{y \; w^a \quad r_b \quad f}{s_e \quad e_e \quad f}$ *constitution.*

f	*y* *w*^{a} *r*$_b$ *f*	*s*$_e$ *e*$_e$ *f*	*y* *w*^{a} *f*	*y* *f*	*w*^{a} *r*$_b$ *f*	*r*$_b$ *f*	*s*$_e$ *f*	*e*$_e$ *f*	Total
45	8	14	0	2	1	0	0	1	71

TABLE 10

Exceptional daughters from attached-X females of $\frac{y \qquad f}{s_e \qquad f}$ *constitution.*

f	*y* *f*	*s*$_e$ *f*	Total
2172	668	627	3467

TABLE 11

Exceptional daughters from attached-X females of $\frac{y \quad e_e \; r_b \quad +}{s_e \; w^a \qquad f}$ *constitution.**

+	*y* *e*$_e$ *r*$_b$	*s*$_e$ *w*^{a}	*y* *e*$_e$	*y*	*e*$_e$ *r*$_b$	*r*$_b$	*s*$_e$	*w*^{a}	*f*	*y* *e*$_e$ *r*$_b$ *f*	*e*$_e$ *r*$_b$ *f*	*s*$_e$ *w*^{a} *f*	*s*$_e$ *f*	*w*^{a} *f*	Total
508	129	195	1	31	25	10	10	12	34	9	4	4	1	3	976

* It is impossible to tell which chromosome contains the mutant factor forked (*f*) and which contains the normal allelomorph.

TABLE 12

Exceptional daughters from attached-X females of $\frac{y \quad r_b \quad +}{s_c \; w^a \quad e_c \quad f}$ *constitution.*

+	y r_b	s_c w^a e_c	y	r_b	s_c w^a	s_c	w^a e_c	e_c	w^a	f	y r_b f	r_b f	s_n w^a e_c f	s_c w^a f	s_c f	w^a e_c f	e_c f	Total
1018	287	278	54	54	35	28	17	31	1	46	12	8	8	2	1	2	1	1883

TABLE 13

Exceptional daughters from attached-X females of $\frac{y \; w^a \quad e_c \quad +}{s_c \quad r_b \quad f}$ *constitution.*

+	y w^a e_c	s_c r_b	y w^a	y	w^a e_c	e_c	s_c	r_b	f	y w^a e_c f	e_c ff	s_c r_b f	s_c f	r_b f	Total
273	87	53	10	4	10	10	17	17	15	2	1	1	2	3	505

TABLE 14

Exceptional daughters from attached-X females of $\frac{y \quad +}{s_c \; w^a \quad e_c \; r_b \quad f}$ *constitution.*

+	y	s_c w^a e_c r_b	s_c w^a	s_c	w^a e_c r_b	e_c r_b	r_b	f	r_b f	s_c f	s_c w^a e_c r_b f	Total
74	30	11	3	2	1	2	3	8	3	1	1	139

TABLE 15

Exceptional daughters from attached-X females of $\frac{y \quad e_c \quad +}{s_c \; w^a \quad r_b \quad f}$ *constitution.*

+	y e_c	s_c w^a r_b	y	e_c	s_c w^a	s_c	w^a r_b	r_b	f	r_b f	Total
86	32	21	2	2	4	2	2	4	6	1	162

THE CONSTITUTION OF PRIMARY EXCEPTIONS OBTAINED AFTER X-RAY TREATMENT OF DROSOPHILA[1]

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INTRODUCTION

The work of MAVOR (1924) on the effect of X-ray treatment of *Drosophila melanogaster* has established a method of artificially increasing the frequency of primary non-disjunction. Primary non-disjunction gives an opportunity to identify two chromosomes coming from the same maturation process. Since the increased frequency due to X-ray treatment makes the accumulation of data on primary non-disjunction less laborious, the experiment herein reported was planned to make use of this agency in the further analysis of the mechanism of crossing over. This experiment is one of a coordinated series of studies on the mechanism of crossing over of which there are already published studies on crossing over in triploids (BRIDGES and ANDERSON 1925) in cases of attached X chromosomes (L. V. MORGAN 1925, ANDERSON 1925) and in secondary exceptions from a high non-disjunction line (ANDERSON 1929).

These studies have already shown that crossing over occurs in a four strand stage and, further, that crossing over regularly involves only two of the four strands at any one level. The usual amount of crossing over occurs even when the chromosomes become permanently attached. Assortment of chromosomes in these cases is regular at the right or spindle-fiber end, being determined presumably by the spindle-fiber attachment. Assort-

[1] Paper from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 297, reporting research conducted by the author while holding appointment as National Research Fellow in Biology.

ment becomes more and more random as we proceed toward the left or distal end, reaching approximate randomness about 40 units to the left of the spindle-fiber attachment.

PLAN OF EXPERIMENT

Echinus cut[6] garnet[2] ($e_c\ c_t\ g$) females were mated to scute crossveinless vermilion forked ($s_c\ c_v\ v\ f$) males. Virgin F_1 females were X-rayed within 28 hours after emergence. The X-ray treatments were administered at the UNIVERSITY OF MICHIGAN hospital. The flies were exposed for ten minutes at a distance of seven inches from the target, the tube being operated at a potential of about 70 kilovolts and a current of 5 milliamperes. Some groups of cultures were treated at about 130 kilovolts, the time of exposure being reduced somewhat. After treatment the flies were mated to yellow scute crossveinless bar ($y\ s_c\ c_v\ B$) or yellow forked bar ($y\ f\ B$) males.

Among the progeny of the rayed females, the regular offspring are bar females and not-bar males. The males show combinations of the recessive characters which were heterozygous in the mother. The exceptional offspring, due to primary non-disjunction, consist of not-bar females and yellow bar ($y\ s_c\ c_v\ B$ or $y\ f\ B$) males. These males are sterile. The exceptional females were mated to yellow scute crossveinless bar ($y\ s_c\ c_v\ B$) males to determine the genetic composition of both their X chromosomes. These two X chromosomes have both been derived from the egg and therefore came from a single maturation process.

A brief report dealing with the frequency of primary non-disjunction after X-ray treatment was presented before the MICHIGAN ACADEMY OF SCIENCE, ARTS, AND LETTERS in 1924 and a report on the proportion of secondary exceptions among the offspring of these primary exceptions was presented before the same academy in 1925 (ANDERSON 1924, 1925b).

FREQUENCY OF PRIMARY NON-DISJUNCTION AFTER X-RAY TREATMENT

In the course of these studies tabulations have been made for 57,877 offspring from X-rayed mothers. These are distributed as follows:

29716	regular (bar) females
27417	regular (not-bar) males
113	exceptional (not-bar) females
631	exceptional (bar) males

These numbers include those reported earlier (ANDERSON 1924). The percentages of exceptions are 0.4 for females and 2.3 for males.

Several hundred of the exceptional males were put up in cultures with virgin females to test for fertility. From one group of cultures a few of the exceptional males were fertile, indicating the presence of some secondary non-disjunction. This group of cultures are not included in the tabulations above. No other exceptional males tested gave offspring.

PHENOTYPIC DISTRIBUTION OF EXCEPTIONS

A total of 195 exceptional females, including the 113 listed above, were classified according to the presence of recessive characteristics which were heterozygous in the parents. Progeny were obtained from 95 of these. The remaining 100 failed to give offspring. Some died before adequate tests were made. Others were continued through several successive culture bottles and proved sterile. The distribution of phenotypes is given in table 1. Those which failed to give offspring are recorded separately from those

TABLE 1

Phenotypic distribution of primary exceptions following X-ray treatment.

PHENOTYPE	FERTILE	WITHOUT OFFSPRING	TOTAL
+	64	53	117
sc	4	4	8
sc cv	6	4	10
sc cv v	3	3	6
sc cv v f	2	3	5
cv v f	2	..	2
v f	3	..	3
f	1	2	3
ec	1	8	9
ec ct	3	5	8
ec ct g	..	3	3
ct g	1	4	5
g	2	3	5
v	1	6	7
cv v	..	2	2
sc v f	1	..	1
ec v f	1	..	1
Total	95	100	195

which gave progeny and whose genotypic constitution was determined. A somewhat larger percentage of wild-type exceptions gave offspring, which may indicate only the greater viability of normal females as contrasted with those which are recessive for the characters involved, or it may indicate a higher percentage of sterility among equational exceptions than among non-equationals. It is possible that some of those recorded as echi-

nus were really triploid intersexes. It is also possible that a few of the fertile wild-type exceptions were secondary exceptions, since one test culture of exceptional males proved fertile.

Table 2 gives the frequency of homozygosis of each of the seven recessive characters which were heterozygous in the X-rayed mothers. The $s_c\ c_v\ v\ f$ chromosome became homozygous in the equationals more frequently than the $e_c\ c_t\ g$ chromosome. As the frequencies are rather low, this

TABLE 2

Frequency of homozygosis of recessive genes.

GENE	FERTILE	WITHOUT OFFSPRING	TOTAL	PERCENT
s_c	16	14	30	15.0
e_c	5	16	21	10.8
c_v	13	12	25	12.8
c_t	4	12	16	8.2
v	13	14	27	13.8
g	3	10	13	6.7
f	10	5	15	7.7

may have no significance. A more striking feature is the relatively high frequency of homozygosis of garnet and forked as contrasted with the low frequencies obtained for attached X chromosomes (ANDERSON 1925a, STURTEVANT 1930), triploids (BRIDGES and ANDERSON 1925), and exceptions from high non-disjunction (ANDERSON 1929).

GENOTYPIC CONSTITUTION OF EXCEPTIONS

Progeny tests were obtained on ninety-five of the exceptions. The genotypic constitutions of these exceptions are listed in table 3. In addition to the genotypic formula, a schematic representation of the constitution is also given in which a is used to represent any gene from the $s_c\ c_v\ v\ f$ parental chromosome, and b any gene from the $e_c\ c_t\ g$ parental chromosome.

TABLE 3

Genotypic constitution of primary exceptions following X-ray treatment.

PHENOTYPE	GENOTYPE	SCHEMATIC REPRESENTATION	CROSSING OVER	FREQUENCY
+	$\frac{s_c\ c_v\ v\ f}{e_c\ c_t\ g}$	$\frac{a\,a\,a\,a\,a\,a\,a}{b\,b\,b\,b\,b\,b\,b}$	0-0	48
+	$\frac{e_c\ c_v\ v\ f}{s_c\ \ c_t\ g}$	$\frac{b\,b\,a\,a\,a\,a\,a}{a\,a\,b\,b\,b\,b\,b}$	2-2	5
+	$\frac{e_c\ c_t\ v\ f}{s_c\ c_v\ \ g}$	$\frac{b\,b\,b\,b\,a\,a\,a}{a\,a\,a\,a\,b\,b\,b}$	4-4	2

TABLE 3 (*continued*)

PHENOTYPE	GENOTYPE	SCHEMATIC REPRESENTATION	CROSSING OVER	FREQUENCY
+	$\frac{e_c\ c_t\ f}{s_c\ c_v\ v\ g}$	$\frac{b\ b\ b\ b\ b\ a\ a}{a\ a\ a\ a\ a\ b\ b}$	5–5	1
+	$\frac{e_c\ c_t\ g\ f}{s_c\ c_v\ v}$	$\frac{b\ b\ b\ b\ b\ b\ a}{a\ a\ a\ a\ a\ a\ b}$	6–6	2
+	$\frac{e_c\ c_t\ v}{s_c\ c_v\ g\ f}$	$\frac{b\ b\ b\ b\ a\ a\ b}{a\ a\ a\ a\ b\ b\ a}$	4, 6–4, 6	1
+	$\frac{c_v\ v\ f}{e_c\ c_t\ g}$	$\frac{b\ a\ a\ a\ a\ a\ a}{b\ b\ b\ b\ b\ b\ b}$	1–0	2
+	$\frac{s_c\ c_c\ v}{e_c\ c_t\ g}$	$\frac{a\ a\ a\ a\ a\ a\ b}{b\ b\ b\ b\ b\ b\ b}$	6–0	2
+	$\frac{s_c\ c_v\ f}{e_c\ c_t\ g}$	$\frac{a\ a\ a\ a\ b\ a\ a}{b\ b\ b\ b\ b\ b\ b}$	4, 5–0	1
s_c	$\frac{s_c\ c_v\ v\ f}{s_c\ e_c\ c_t\ g}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ b\ b\ b\ b\ b\ b}$	0–1	1
s_c	$\frac{s_c\ c_v\ v\ f}{s_c\ c_t\ g}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ a\ b\ b\ b\ b\ b}$	0–2	2
s_c	$\frac{s_c\ c_v\ g}{s_c\ e_c\ c_t\ v\ f}$	$\frac{a\ a\ a\ a\ b\ b\ b}{a\ b\ b\ b\ a\ a\ a}$	4–1, 4	1
$s_c\ c_v$	$\frac{s_c\ c_v\ v\ f}{s_c\ c_v\ c_t\ g}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ a\ a\ b\ b\ b\ b}$	0–3	1
$s_c\ c_v$	$\frac{s_c\ c_v\ v\ f}{s_c\ c_v\ g}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ a\ a\ a\ b\ b\ b}$	0–4	4
$s_c\ c_v$	$\frac{s_c\ c_v\ v}{s_c\ c_v\ c_t\ g\ f}$	$\frac{a\ a\ a\ a\ a\ a\ b}{a\ a\ a\ b\ b\ b\ a}$	6–3, 6	1
$s_c\ c_v\ v$	$\frac{s_c\ c_v\ v\ f}{s_c\ c_v\ v\ g}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ a\ a\ a\ \dot{a}\ b\ b}$	0–5	3
$s_c\ c_v\ v\ f$	$\frac{s_c\ c_v\ v\ f}{s_c\ c_v\ v\ f}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ a\ a\ a\ a\ a\ a}$	0–0	2
$c_v\ v\ f$	$\frac{s_c\ c_v\ v\ f}{e_c\ c_v\ v\ f}$	$\frac{a\ a\ a\ a\ a\ a\ a}{b\ b\ a\ a\ a\ a\ a}$	0–2	2
$v\ f$	$\frac{s_c\ c_v\ v\ f}{e_c\ c_t\ v\ f}$	$\frac{a\ a\ a\ a\ a\ a\ a}{b\ b\ b\ b\ a\ a\ a}$	0–4	1
$v\ f$	$\frac{s_c\ c_v\ v\ f}{e_c\ v\ f}$	$\frac{a\ a\ a\ a\ a\ a\ a}{b\ b\ b\ a\ a\ a\ a}$	0–3	1
$v\ f$	$\frac{c_v\ v\ f}{s_c\ e_c\ c_t\ v\ f}$	$\frac{b\ a\ a\ a\ a\ a\ a}{a\ b\ b\ b\ a\ a\ a}$	1–1, 4	1
f	$\frac{s_c\ c_v\ v\ f}{e_c\ c_t\ f}$	$\frac{a\ a\ a\ a\ a\ a\ a}{b\ b\ b\ b\ b\ a\ a}$	0–5	1
e_c	$\frac{e_c\ c_t\ v\ f}{e_c\ c_v\ g}$	$\frac{b\ b\ b\ b\ a\ a\ a}{b\ b\ a\ a\ b\ b\ b}$	4–2, 4	1

TABLE 3 (*continued*)

PHENOTYPE	GENOTYPE	SCHEMATIC REPRESENTATION	CROSSING OVER	FREQUENCY
$e_c\ c_t$	$\frac{e_c\ c_t\ v\ f}{e_c\ c_t\ g}$	$\frac{b\ b\ b\ b\ a\ a\ a}{b\ b\ b\ b\ b\ b\ b}$	4–0	3
g	$\frac{s_c\ c_v\ g}{e_c\ c_t\ g}$	$\frac{a\ a\ a\ a\ b\ b\ b}{b\ b\ b\ b\ b\ b\ b}$	4–0	2
$c\ g$	$\frac{s_c\ c_t\ g\ f}{e_c\ c_t\ g}$	$\frac{a\ a\ b\ b\ b\ b\ a}{b\ b\ b\ b\ b\ b\ b}$	2, 6–0	1
v	$\frac{s_c\ c_v\ v\ f}{e_c\ v\ g}$	$\frac{a\ a\ a\ a\ a\ a\ a}{b\ b\ b\ a\ a\ b\ b}$	0–3, 5	1
$s_c\ v\ f$	$\frac{s_c\ c_v\ v\ f}{s_c\ c_t\ v\ f}$	$\frac{a\ a\ a\ a\ a\ a\ a}{a\ a\ b\ b\ a\ a\ a}$	0–2, 4	1
$e_c\ v\ f$	$\frac{s_c\ e_c\ c_v\ v\ f}{e_c\ c_t\ v\ f}$	$\frac{a\ b\ a\ a\ a\ a\ a}{b\ b\ b\ b\ a\ a\ a}$	1, 2–4	1

From the data of table 3 the frequency of homozygosis for each locus can be tabulated. This has the advantage over the phenotypic distributions in that the frequencies of homozygosis of the normal allelomorphs may be tabulated as well as that of the recessive genes. These frequencies are presented in table 4. The percentages are computed on the basis of 190

TABLE 4

Frequency of homozygosis for single loci summarized from table 3.

LOCUS	FREQUENCY	PERCENTAGE
s_c	22	11.6
e_c	19	10.0
c_v	17	9.0
c_t	17	9.0
v	17	9.0
g	13	7.0
f	14	7.4

instead of 95 to make them comparable with the percentages calculated for the phenotypic distributions. These percentages are more uniform than those of table 2, due to their not being influenced by the unequal frequency of homozygosis of the two chromosomes. A combination of the data from table 2, on the exceptions which gave no offspring, with the data of table 4 gives the following percentages of exceptions:

s_c—12.4	v—10.6
e_c—12.0	g— 7.9
c_v—10.0	f— 6.6
c_t—10.0	

The data on crossing over from table 3 are summarized in table 5. The control percentages given for comparison are the normal percentages of

TABLE 5

Summary of data on crossing over from table 3.

REGION	PERCENTAGE OF CROSSING OVER	CONTROL	RATIO TO CONTROL
$s_c - e_s$	3.7	6.8	.54
$e_c - c_v$	9.5	9.7	.98
$c_v - c_t$	2.1	8.4	.25
$c_t - v$	12.6	14.8	.85
$v - g$	4.3	11.2	.38
$g - f$	5.8	11.3	.51
Total	38.0	62.2	.61

crossing over shown by the same parental stocks (ANDERSON 1929). The observed percentages of crossing over in the exceptions are irregular but are all lower than the controls. The total crossing over is about sixty percent of the normal.

ASSOCIATION OF CROSSOVERS

When only a single region is considered, a crossover may be associated with a non-crossover or another crossover. In the former case, either the left or the right side may be homozygous, that is, it may be *ab/aa* or *ba/aa*. When two crossovers are associated they may be complementary *ab/ba* or identical *ab/ab*. No case was observed of association of identical crossovers. This is further evidence that only two out of the four strands cross over at any one level.

The data on association of crossovers in single regions are summarized in table 6. There were 40 combinations of a crossover with a non-crossover to 16 combinations of complementary crossovers. This approaches the four to one ratio expected for random assortment.

TABLE 6

Association of crossovers.

REGION	TYPE OF ASSOCIATION		
	ab / *aa*	*ba* / *aa*	*ab* / *ba*
1	4	1	1
2	5	3	5
3	2	2	0
4	7	7	5
5	5	1	1
6	1	2	4
Total	24	16	16

The rightmost crossovers are summarized in table 7. All cases where the *f* locus was homozygous are excluded. Among the rightmost crossovers there were only 19 *ab/aa* combinations to 14 *ab/ba* combinations. The data on attached X chromosomes (ANDERSON 1925a, STURTEVANT 1930) and on high non-disjunction (ANDERSON 1929) gave close approximations to the expected two to one ratio.

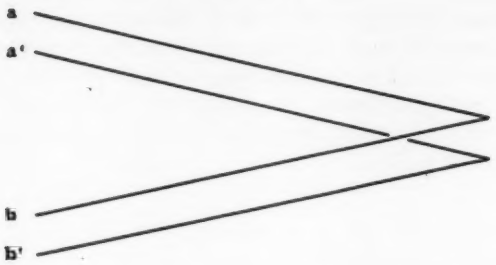
TABLE 7

Association of rightmost crossovers

REGION	TYPE OF ASSOCIATION	
	ab / *aa*	*ab* / *ba*
1	3	0
2	2	5
3	1	0
4	7	4
5	5	1
6	1	4
Total	19	14

INTERPRETATION

The data obtained on the X constitution of primary exceptions from X-ray treatment resemble in general the data on crossing over in attached-X cases (ANDERSON 1925a, STURTEVANT 1930) and on crossing over among the exceptions from high non-disjunction (ANDERSON 1929). One striking difference is noted, which calls for special consideration. This is the relatively high frequency of homozygosis of garnet and forked. In the attached-X and high non-disjunction cases the percentages of homozygosis were very low near the right or spindle-fiber end of the chromosome, converging toward zero at about 10 or 12 units beyond forked, a point which is believed to represent the end of the chromosome. This point practically coincides with the mapped position of bobbed, the rightmost known gene in the X chromosome. STERN (1926) has given independent evidence that the position of the spindle-fiber attachment is very close to the locus of bobbed. The absence of homozygosis at the spindle-fiber end in the attached-X case shows that distribution is regular at that point, being presumably determined by the spindle-fiber attachment. The attachment of the chromosomes prevents any separation at the reductional division. The random proportion of one reciprocal *ab/ba* to two equational *ab/aa* combinations among rightmost crossovers shows that of the four strands indicated in the diagram, *a* must cross over equally freely with each of the two



sister strands b and b'. This is further corroborated by the frequencies of homozygosis regularly increasing toward the left. For loci near the spindle-fiber end, the percentage of homozygosis approaches the calculated value of one-half of the map distance from the spindle-fiber end. Proceeding toward the left, it drops below this value due to multiple crossing over. At about the middle of the chromosome map, it approaches close to 16.7 percent, the percentage expected for random assortment. Since the percentage of crossing over is not altered by the attachment of the chromosomes, it is very probable that the mechanism of crossing over in these cases is the normal mechanism.

The exceptions from the high non-disjunction line showed the same decreasing frequency of homozygosis toward the right and the same random proportion between reciprocal and equational combinations of rightmost crossovers. These relations indicate that the non-disjunction occurs always at the reductional division while the behavior at the equational division remains normal, just as in the attached-X cases. The percentage of crossing over is much lower than among the regular offspring, especially for regions near the spindle fiber. However this only indicates that non-disjunction rarely occurs when the two X chromosomes have been intimately synapsed in the neighborhood of the spindle-fiber attachment. But when non-disjunction occurs the behavior is the same as in the attached-X cases.

Among the primary exception induced by X-ray treatment the percentages of homozygosis do not converge toward zero at the spindle-fiber attachment. In fact the data presented (tables 2 and 4) indicate that more than ten percent of these exceptions were homozygous for the rightmost end and of one or the other of the two parental chromosomes. The interpretation which seems most logical, is that these were due to non-disjunction occurring at the equational division. Exceptions formed in this way should be homozygous at the spindle-fiber end, and this homozygosis should only be disturbed where one of the two sister strands has crossed

over with a strand from the opposite chromosome. The curve of homozygosis for equational division exceptions should be the reciprocal of the curve for reductional division exceptions or for attached-X progenies. Homozygosis should be 100 percent at the spindle-fiber end, decreasing toward the left until the random expectation of 16.7 percent is reached. In a distribution including both kinds of exceptions, the effect of equational division exceptions would be most evident near the spindle-fiber end of the curve.

SUMMARY

Virgin females of constitution $s_c\ c_v\ v\ f/e_c\ c_t\ g$ were subjected to X-rays and mated to bar males. The exceptional daughters produced were bred to determine their X constitution.

Of the females 0.4 percent were exceptional, of the males 2.3 percent.

The chromosomes going into exceptional (XX) eggs showed about 60 percent of the normal amount of crossing over.

The percentages of homozygosis were as follows:

s_c—12.4
e_c—12.0
c_v—10.0
c_t—10.0
v—10.6
g— 7.9
f— 6.6

The comparatively high values for homozygosis of garnet and forked are believed to be due to non-disjunction occurring at both maturation divisions after X-ray treatment.

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Organized by the Italian Committee for the Study of Population Problems, an International Congress for Studies Regarding Population, will be held in Rome from 7th to 10th September next, under the Honorary Chairmanship of H. E. BENITO MUSSOLINI and the effective Chairmanship of Professor CORRADO GINI, President of the aforesaid Italian Committee. The Congress will include: (a) An Honorary Committee. (b) An International Committee of Patronage, composed of presidents and representatives of national scientific organizations for the study of population problems, and other persons. (c) An Organizing Committee, composed of Italian members will be entrusted with the organization of the Congress and will be responsible for the acceptance of communications.

The program of the Congress is scientific, so that political, moral, or religious propaganda is forbidden, even if questions concerning the problems of population are involved.

The Congress will consist of the following Sections: Biology and Eugenics, Anthropology and Geography, Hygiene and Medicine, Demography, Economy, Sociology, History, Methodology. English, French, German, Italian and Spanish, will be the official languages of the Congress.

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Some subjects of special importance, chosen by the Organizing Committee, will be the object of reports by persons previously nominated.

The fee entitling members to take part in the Congress and to receive a copy of the Proceedings is: 50 lire for those invited and 100 Lire for all others.

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